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(54) Title: 3, 11B CIS DIHYDROTETRABENAZINE FOR THE TREATMENT OF SCHIZOPHRENIA AND OTHER PSYCHOSES

(57) Abstract: The invention provides the use of 3,11 b-*cis*-dihydrotetrabenazine or a pharmaceutically acceptable salt thereof for the manufacture of a medicament for the prophylaxis or treatment of schizophrenia and other psychoses.

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PHARMACEUTICAL COMPOUNDS

This invention relates to the use of dihydrotetrabenazine in the prophylaxis or treatment of a psychosis.

Background of the Invention

Psychosis is a generic psychiatric term for mental states in which the components of rational thought and perception are severely impaired. Persons experiencing a psychosis may experience hallucinations, hold paranoid or delusional beliefs, demonstrate personality changes and exhibit disorganized thinking. This is usually accompanied by a lack of insight into the unusual or bizarre nature of their behavior, difficulties with social interaction and impairments in carrying out the activities of daily living. Essentially, a psychotic episode involves loss of contact with reality.

Psychosis is often considered to be a symptom of severe mental illness. Although it is not exclusively linked to any particular psychological or physical state, it is particularly associated with schizophrenia, bipolar disorder (manic depression) and severe clinical depression. There are also several physical circumstances that can induce a psychotic state, including electrolyte disorder, urinary tract infections in the elderly, pain syndromes, drug toxicity, and drug withdrawal (especially alcohol, barbiturates, and sometimes benzodiazepines), as well as infections of or injuries to the brain (these psychoses are now more commonly referred to as organic mental disorders).

Psychosis may be caused by or follow brain injury and may occur after drug use, particularly after drug overdose, chronic use, and during drug withdrawal.

Chronic psychological stress is also known to cause psychotic states, although the exact mechanism by which this occurs is uncertain. Short-lived psychosis triggered by stress is known as brief reactive psychosis.

Psychotic episodes can be significantly coloured by mood. For example, people experiencing a psychotic episode in the context of depression may experience persecutory or self-blaming delusions or hallucinations, whilst people experiencing

a psychotic episode in the context of mania may form grandiose delusions or have an experience of deep religious significance.

Hallucinations are defined as sensory perception in the absence of external stimuli. Psychotic hallucinations may occur in any of the five senses and take on almost any form, which may include simple sensations (such as lights, colours, tastes, smells) to more meaningful experiences such as seeing and interacting with fully formed animals and people, hearing voices and complex tactile sensations.

Auditory hallucinations, particularly the experience of hearing voices, are a common and often prominent feature of psychosis. Hallucinated voices may talk about, or to, the person, and may involve several speakers with distinct personas. Auditory hallucinations tend to be particularly distressing when they are derogatory, commanding or preoccupying.

Psychosis may involve delusional or paranoid beliefs. Psychotic delusions can be classified into primary and secondary types. Primary delusions are defined as arising out-of-the-blue and not being comprehensible in terms of normal mental processes, whereas secondary delusions may be understood as being influenced by the person's background or current situation.

Thought disorder describes an underlying disturbance to conscious thought and is classified largely by its effects on speech and writing. Affected persons may show pressure of speech (speaking incessantly and quickly), derailment or flight of ideas (switching topic mid-sentence or inappropriately), thought blocking, rhyming or punning.

One important and poorly understood feature of psychosis is usually an accompanying lack of insight into the unusual, strange or bizarre nature of the person's experience or behaviour. Even in the case of an acute psychosis, sufferers may seem completely unaware that their vivid hallucinations and impossible delusions are in any way unrealistic. However, insight can vary between individuals and throughout the duration of the psychotic episode. In some cases, particularly with auditory and visual hallucinations, the patient has good insight and this makes

the psychotic experience even more terrifying in that the patient realizes that he or she should not be hearing voices, but does.

There are a number of possible causes for psychosis. Psychosis may be the result of an underlying mental illness such as Bipolar disorder (also known as manic depression), and schizophrenia. Psychosis may also be triggered or exacerbated by severe mental stress and high doses or chronic use of drugs such as amphetamines, LSD, PCP, cocaine or scopolamine. Sudden withdrawal from CNS depressant drugs, such as alcohol and benzodiazepines, may also trigger psychotic episodes. As can be seen from the wide variety of illnesses and conditions in which psychosis has been reported to arise (including for example, AIDS, leprosy, malaria and even mumps) there is no singular cause of a psychotic episode.

Schizophrenia is the name given to a group of psychotic disorders usually characterized by withdrawal from reality, illogical patterns of thinking, delusions, and hallucinations, and accompanied in varying degrees by other emotional, behavioral, or intellectual disturbances. Schizophrenia is associated with dopamine imbalances in the brain and defects of the frontal lobe and is caused by genetic and other biological factors and psychosocial factors.

The drugs traditionally used to treat psychoses such as those associated with schizophrenia (the so-called "typical" antipsychotics) effectively control the hallucinations, delusions, and confusion associated with these conditions. Such drugs, examples of which include haloperidol, chlorpromazine, and fluphenazine, have been available since the mid-1950s. These drugs act primarily by blocking dopamine receptors and are effective in treating the "positive" symptoms of psychosis.

Four major areas of the brain are involved as primary pathways for dopamine. They include the nigrostriatal, mesocortical, mesolimbic, and tuberoinfundibular systems. Decreased dopamine activity in the mesocortical tract (as seen in the schizophrenic patient) results in an inability for the prefrontal areas of the brain to activate. Positive symptoms, such as hallucinations and delusions, can occur when overactivity of dopamine in the mesolimbic tract occurs. There are five subcategories of dopamine receptors in the brain. Conventional antipsychotics have

the greatest impact on the D2 receptor. The so-called "atypical" antipsychotic agents (see below) typically have a weaker effect on D2 receptors with more potent blockade on the D4 receptor which is mostly found in the frontal cortex and the hippocampus.

Conventional ("typical") antipsychotics block D2 receptors nonselectively in all four areas of the brain. The resulting effect in the mesolimbic tract reduces hallucinations and delusions. However, a concurrent reduction in dopamine in the nigrostriatal pathway can produce extrapyramidal symptoms. Blockade of dopamine may also worsen negative symptoms and cognitive functioning by further decreasing the amount of dopamine in the frontal cortex. The tuberoinfundibular tract is affected by all the conventional antipsychotics, which may cause neuroendocrine and hypothalamic dysfunction. Dopamine blockage in the tuberoinfundibular tract is responsible for increases in prolactin levels.

Thus, the use of "typical" anti-psychotics is associated with a number of undesirable side effects.

The atypical antipsychotics target the limbic area more specifically when blocking dopamine D2 receptors. Consequently, they have less impact on the nigrostriatal and mesocortical pathways, resulting in a reduced potential for adverse effects. As noted earlier, they also tend to have a greater affinity for dopamine D4 receptors.

The receptor binding profiles of atypical antipsychotic drugs is reviewed in the article by A. E. Hensiek & M. R. Trimble, *J. Neurology, Neurosurgery and Psychiatry*, (2002), 72:281-285.

The newer "atypical" antipsychotics – often referred to as the serotonin-dopamine antagonists (SDAs) - block both serotonin and dopamine receptors, thereby treating both the "positive" and "negative" symptoms of schizophrenia – see H. Y. Meltzer, *J. Clin. Psychopharmacol.* (1995), Feb;15(1 Suppl 1):2S-3S and M. Huttunen,. *J. Clin. Psychopharmacol.* (1995), Feb;15(1 Suppl 1):4S-10S. These newer medications are effective in treating a broader range of symptoms of psychosis and schizophrenia, and have fewer side effects than traditional antipsychotics. For

example, they have a lower propensity than typical antipsychotics to cause extrapyramidal side effects and prolactin elevation.

Examples of these newer atypical antipsychotics (the "serotonin-dopamine antagonists") include clopazine, risperidone, asenapine, olanzapine and iloperidone.

Tetrabenazine (Chemical name: 1, 3, 4,6,7,11b-hexahydro-9,10-dimethoxy-3-(2-methylpropyl)-2H-benzo(a)quinolizin-2-one) has been in use as a pharmaceutical drug since the late 1950s. Initially developed as an anti-psychotic, tetrabenazine is currently used in the symptomatic treatment of hyperkinetic movement disorders such as Huntington's disease, hemiballismus, senile chorea, tic, tardive dyskinesia and Tourette's syndrome, see for example Jankovic *et al.*, *Am. J. Psychiatry.* (1999) Aug; 156(8):1279-81 and Jankovic *et al.*, *Neurology* (1997) Feb; 48(2):358-62.

The chemical structure of tetrabenazine is as shown in Figure 1 below.

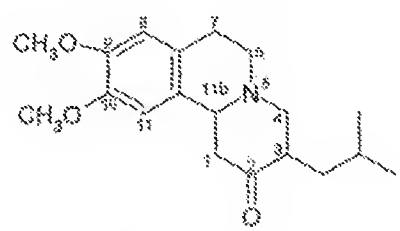


Figure 1- Structure of tetrabenazine

The compound has chiral centres at the 3 and 11b carbon atoms and hence can, theoretically, exist in a total of four isomeric forms, as shown in Figure 2.

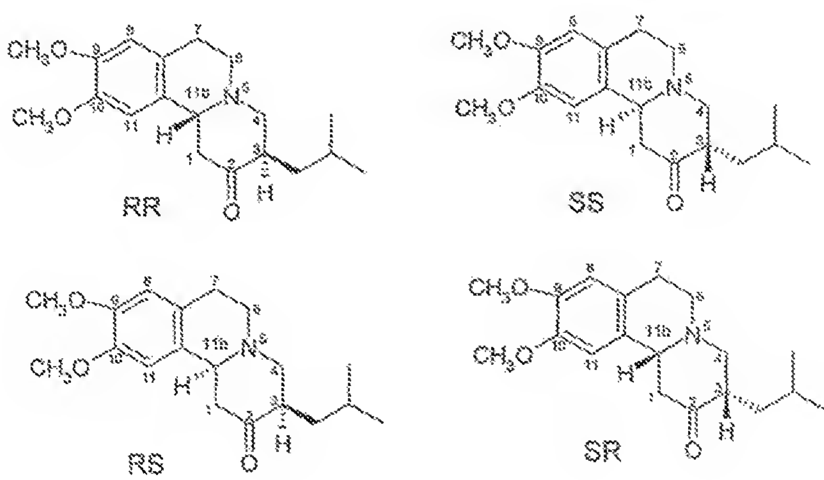


Figure 2 – Possible tetrabenazine isomers

In Figure 2, the stereochemistry of each isomer is defined using the "R and S" nomenclature developed by Cahn, Ingold and Prelog, see *Advanced Organic Chemistry* by Jerry March, 4th Edition, John Wiley & Sons, New York, 1992, pages 109-114. In Figure 2 and elsewhere in this patent application, the designations "R" or "S" are given in the order of the position numbers of the carbon atoms. Thus, for example, *RS* is a shorthand notation for 3*R*,11b*S*. Similarly, when three chiral centres are present, as in the dihydrotetrabenazines described below, the designations "*R*" or "*S*" are listed in the order of the carbon atoms 2, 3 and 11b. Thus, the *2S,3R,11bR* isomer is referred to in short hand form as *SRR* and so on.

Commercially available tetrabenazine is a racemic mixture of the *RR* and *SS* isomers and it would appear that the *RR* and *SS* isomers (hereinafter referred to individually or collectively as *trans*-tetrabenazine because the hydrogen atoms at the 3 and 11b positions have a *trans* relative orientation) are the most thermodynamically stable isomers.

Tetrabenazine has somewhat poor and variable bioavailability. It is extensively metabolised by first-pass metabolism, and little or no unchanged tetrabenazine is typically detected in the urine. The major metabolite is dihydrotetrabenazine (Chemical name: 2-hydroxy-3-(2-methylpropyl)-1,3,4,6,7,11b-hexahydro-9,10-dimethoxy-benzo(a)quinolizine) which is formed by reduction of the 2-keto group in tetrabenazine, and is believed to be primarily responsible for the activity of the drug (see Mehvar *et al., Drug Metab.Disp*, 15, 250-255 (1987) and *J. Pharm. Sci.*, 76, No.6, 461-465 (1987)).

Four dihydrotetrabenazine isomers have previously been identified and characterised, all of them being derived from the more stable *RR* and *SS* isomers of the parent tetrabenazine and having a *trans* relative orientation between the hydrogen atoms at the 3 and 11b positions) (see Kilbourn *et al., Chirality*, 9:59-62 (1997) and Brossi *et al., Helv. Chim. Acta.*, vol. XLI, No. 193, pp1793-1806 (1958). The four isomers are (+)-α-dihydrotetrabenazine, (-)-α-dihydrotetrabenazine, (+)-β-dihydrotetrabenazine and (-)-β-dihydrotetrabenazine. The structures of the four known dihydrotetrabenazine isomers are considered to be as shown in Figure 3.

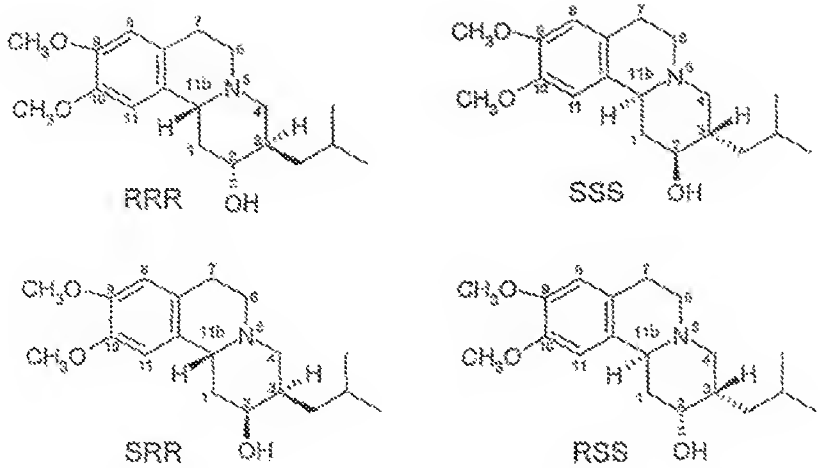


Figure 3 – Structures of known isomers of dihydrotetrabenazine

Kilbourn *et al.*,(see *Eur. J. Pharmacol.*, 278:249-252 (1995) and *Med. Chem. Res.*, 5:113-126 (1994)) investigated the specific binding of individual radio-labelled dihydrotetrabenazine isomers in the conscious rat brain. They found that the (+)-α-[^{11}C]dihydrotetrabenazine (2*R*,3*R*,11b*R*) isomer accumulated in regions of the brain associated with higher concentrations of the neuronal membrane dopamine transporter (DAT) and the vesicular monoamine transporter (VMAT2). However, the essentially inactive (-)-α-[^{11}C]dihydrotetrabenazine isomer was almost uniformly distributed in the brain, suggesting that specific binding to DAT and VMAT2 was not occurring. The *in vivo* studies correlated with *in vitro* studies which demonstrated that the (+)-α-[^{11}C]dihydrotetrabenazine isomer exhibits a K_i for [^{3}H]methoxytetrabenazine >2000-fold higher than the K_i for the (-)-α-[^{11}C]dihydrotetrabenazine isomer.

Our earlier International patent application No. PCT/GB2005/000464 discloses the preparation and use of pharmaceutical dihydrotetrabenazine isomers derived from the unstable *RS* and *SR* isomers (hereinafter referred to individually or collectively as *cis*-tetrabenazine because the hydrogen atoms at the 3 and 11b positions have a *cis* relative orientation) of tetrabenazine.

Summary of the Invention

It has now been found that *cis*-dihydrotetrabenazines described in our earlier application no. PCT/GB2005/000464 demonstrate receptor binding profiles broadly

similar to the receptor binding profiles of atypical antipsychotic agents. In particular, the *cis*-dihydrotetrabenazines exhibit both dopaminergic and serotinergic inhibitory actions. The receptor binding profiles of the *cis*-dihydrotetrabenazines indicate that they will be of use in the prophylaxis or treatment of psychosis, for example psychosis arising from or associated with schizophrenia.

Accordingly, in a first aspect, the invention provides 3, 11b-*cis*-dihydrotetrabenazine for use in the prophylaxis or treatment of psychosis.

In another aspect, the invention provides 3, 11b-*cis*-dihydrotetrabenazine for use in preventing or alleviating psychosis.

In another aspect, the invention provides 3, 11b-*cis*-dihydrotetrabenazine for use in preventing, alleviating or reducing one or more symptoms of schizophrenia.

The invention also provides:

- The use of 3, 11b-*cis*-dihydrotetrabenazine for the manufacture of a medicament for the prophylaxis or treatment of psychosis.
- A method for the prophylaxis or treatment of psychosis, the method comprising administering to the mammal a therapeutically effective amount of *cis*-dihydrotetrabenazine.
- The use of 3, 11b-*cis*-dihydrotetrabenazine for the manufacture of a medicament for preventing or alleviating a psychotic episode.
- A method for preventing or alleviating a psychotic episode, the method comprising administering to the mammal a therapeutically effective amount of *cis*-dihydrotetrabenazine.
- A method or use as defined above wherein the psychosis or psychotic episode arises from or is associated with schizophrenia.
- The use of 3, 11b-*cis*-dihydrotetrabenazine for the manufacture of a medicament for the prophylaxis or treatment of schizophrenia.

- A method for the prophylaxis or treatment of schizophrenia, the method comprising administering to the mammal a therapeutically effective amount of *cis*-dihydrotetrabenazine.

- The use of 3, 11b-*cis*-dihydrotetrabenazine for the manufacture of a medicament for preventing, alleviating or reducing one or more symptoms of schizophrenia.

- A method for preventing, alleviating or reducing one or more symptoms of schizophrenia, the method comprising administering to the mammal a therapeutically effective amount of *cis*-dihydrotetrabenazine.

The psychotic episodes, psychoses or symptoms prevented, alleviated or reduced in accordance with the invention may be any one or more symptoms selected from:

- delusions;
- hallucinations;
- visual hallucinations;
- auditory hallucinations;
- hallucinations involving tactile sensations, tastes or smells;
- confusion;
- emotional, behavioral, or intellectual disturbances;
- withdrawal from reality;
- illogical and/or disorganized patterns of thinking;
- paranoid or delusional beliefs;
- paranoia
- grandiose delusions;
- persecutory or self-blaming delusions; and
- personality changes.

The psychotic episodes, psychoses or symptoms prevented, alleviated or reduced in accordance with the invention may be any one or more selected from those arising from or associated with:

- psychosis caused by or associated with schizophrenia;

- psychosis caused by or associated with bipolar disorder (manic depression);
- psychosis caused by or associated with severe clinical depression;
- psychosis induced by disorders and conditions such as:
    - electrolyte disorder;
    - urinary tract infections in the elderly;
    - pain syndromes;
    - drug toxicity;
    - drug withdrawal; and
    - infections of or injuries to the brain;
- psychosis caused by chronic psychological stress (brief reactive psychosis);
- psychosis triggered or exacerbated by severe mental stress; and
- psychosis triggered by or arising from or following illnesses and conditions such as AIDS, leprosy, malaria and mumps.

In one embodiment, the symptoms or psychoses arise from or are associated with schizophrenia and may be any one or more symptoms selected from:

- delusions;
- hallucinations;
- confusion;
- emotional, behavioral, or intellectual disturbances;
- withdrawal from reality; and
- illogical patterns of thinking.

The *cis*-dihydrotetrabenazine used in the present invention is 3, 11b, *cis*-dihydrotetrabenazine.

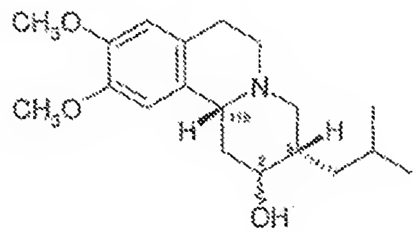
The 3,11b-*cis*-dihydrotetrabenazine used in the invention may be in substantially pure form, for example at an isomeric purity of greater than 90%, typically greater than 95% and more preferably greater than 98%.

The term "isomeric purity" in the present context refers to the amount of 3,11b-*cis*-dihydrotetrabenazine present relative to the total amount or concentration of dihydrotetrabenazine of all isomeric forms. For example, if 90% of the total

dihydrotetrabenazine present in the composition is 3,11b-*cis*-dihydrotetrabenazine, then the isomeric purity is 90%.

The 11b-*cis*-dihydrotetrabenazine used in the invention may be in the form of a composition which is substantially free of 3,11b-*trans*-dihydrotetrabenazine, preferably containing less than 5% of 3,11b-*trans*-dihydrotetrabenazine, more preferably less than 3% of 3,11b-*trans*-dihydrotetrabenazine, and most preferably less than 1% of 3,11b-*trans*-dihydrotetrabenazine.

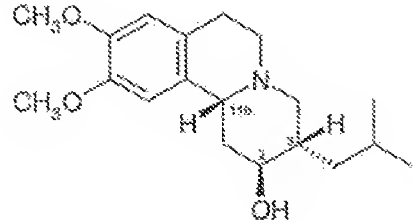
The term "3,11b-*cis*-" as used herein means that the hydrogen atoms at the 3- and 11b-positions of the dihydrotetrabenazine structure are in the *cis* relative orientation. The isomers of the invention are therefore compounds of the formula (I) and antipodes (mirror images) thereof.



(I)

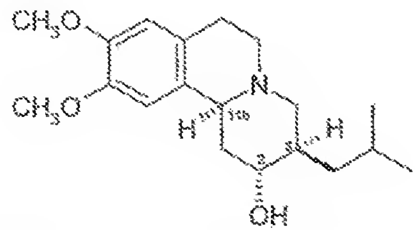
There are four possible isomers of dihydrotetrabenazine having the 3,11b-*cis* configuration and these are the 2*S*,3*S*,11b*R* isomer, the 2*R*,3*R*,11b*S* isomer, the 2*R*,3*S*,11b*R* isomer and the 2*S*,3*R*,11b*S* isomer. The four isomers have been isolated and characterised and, in another aspect, the invention provides the use of individual isomers of 3,11b-*cis*-dihydrotetrabenazine. In particular, the invention provides:

(a) the 2*S*,3*S*,11b*R* isomer of 3,11b-*cis*-dihydrotetrabenazine having the formula (Ia):



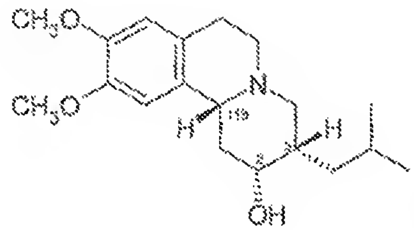
(Ia)

(b) the 2*R*,3*R*,11b*S* isomer of 3,11b-*cis*-dihydrotetrabenazine having the formula (Ib):



(Ib)

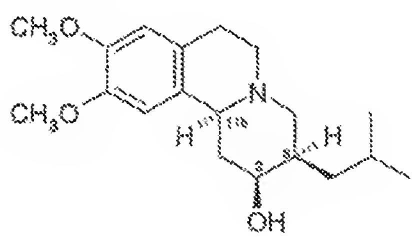
(c) the 2*R*,3*S*,11b*R* isomer of 3,11b-*cis*-dihydrotetrabenazine having the formula (Ic):



(Ic)

and

(d) the 2*S*,3*R*,11b*S* isomer of 3,11b-*cis*-dihydrotetrabenazine having the formula (Id):



The individual isomers of the invention can be characterised by their spectroscopic, optical and chromatographic properties, and also by their absolute stereochemical configurations as determined by X-ray crystallography.

Without implying any particular absolute configuration or stereochemistry, the four novel isomers may be characterised as follows:

<u>Isomer A</u>

Optical activity as measured by ORD (methanol, 21°C): laevorotatory (-)

IR Spectrum (KBr solid), ^{1}H-NMR spectrum ($CDCl_3$) and ^{13}C-NMR spectrum ($CDCl_3$) substantially as described in Table 1.

Isomer B

Optical activity as measured by ORD (methanol, 21°C): dextrorotatory (+)
IR Spectrum (KBr solid), ^{1}H-NMR spectrum ($CDCl_3$) and ^{13}C-NMR spectrum ($CDCl_3$) substantially as described in Table 1, and X-ray crystallographic properties as described in Example 4.

Isomer C

Optical activity as measured by ORD (methanol, 21°C): dextrorotatory (+)
IR Spectrum (KBr solid), ^{1}H-NMR spectrum ($CDCl_3$) and ^{13}C-NMR spectrum ($CDCl_3$) substantially as described in Table 2.

Isomer D

Optical activity as measured by ORD (methanol, 21°C): laevorotatory (-)
IR Spectrum (KBr solid), ^{1}H-NMR spectrum ($CDCl_3$) and ^{13}C-NMR spectrum ($CDCl_3$) substantially as described in Table 2.

ORD values for each isomer are given in the examples below but it is noted that such values are given by way of example and may vary according to the degree of purity of the isomer and the influence of other variables such as temperature fluctuations and the effects of residual solvent molecules.

The enantiomers A, B, C and D may each be presented in a substantially enantiomerically pure form or as mixtures with other enantiomers of the invention.

The terms "enantiomeric purity" and "enantiomerically pure" in the present context refer to the amount of a given enantiomer of 3,11b-*cis*-dihydrotetrabenazine present relative to the total amount or concentration of dihydrotetrabenazine of all enantiomeric and isomeric forms. For example, if 90% of the total dihydrotetrabenazine present in the composition is in the form of a single enantiomer, then the enantiomeric purity is 90%.

By way of example, in each aspect and embodiment of the invention, each individual enantiomer selected from Isomers A, B, C and D may be present in an

enantiomeric purity of at least 55% (e.g. at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, 99.5% or 100%).

The isomers of the invention may also be presented in the form of mixtures of one or more of Isomers A, B, C and D. Such mixtures may be racemic mixtures or non-racemic mixtures. Examples of racemic mixtures include the racemic mixture of Isomer A and Isomer B and the racemic mixture of Isomer C and Isomer D.

Pharmaceutically Acceptable Salts

Unless the context requires otherwise, a reference in this application to dihydrotetrabenazine and its isomers, includes within its scope not only the free base of the dihydrotetrabenazine but also its salts, and in particular acid addition salts.

Particular acids from which the acid addition salts are formed include acids having a pKa value of less than 3.5 and more usually less than 3. For example, the acid addition salts can be formed from an acid having a pKa in the range from +3.5 to -3.5.

Preferred acid addition salts include those formed with sulphonic acids such as methanesulphonic acid, ethanesulphonic acid, benzene sulphonic acid, toluene sulphonic acid, camphor sulphonic acid and naphthalene sulphonic acid.

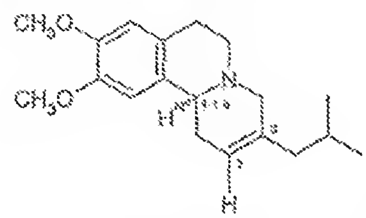
One particular acid from which acid addition salts may be formed is methanesulphonic acid.

Acid addition salts can be prepared by the methods described herein or conventional chemical methods such as the methods described in *Pharmaceutical Salts: Properties, Selection, and Use*, P. Heinrich Stahl (Editor), Camille G. Wermuth (Editor), ISBN: 3-90639-026-8, Hardcover, 388 pages, August 2002. Generally, such salts can be prepared by reacting the free base form of the compound with the appropriate base or acid in water or in an organic solvent, or in a mixture of the two; generally, nonaqueous media such as ether, ethyl acetate, ethanol, isopropanol, or acetonitrile are used.

The salts are typically pharmaceutically acceptable salts. However, salts that are not pharmaceutically acceptable may also be prepared as intermediate forms which may then be converted into pharmaceutically acceptable salts. Such non-pharmaceutically acceptable salt forms also form part of the invention.

Methods for the Preparation of Dihydrotetrabenazine Isomers

The dihydrotetrabenazine of the invention can be prepared by a process comprising the reaction of a compound of the formula (II):



(II)

with a reagent or reagents suitable for hydrating the 2,3-double bond in the compound of formula (II) and thereafter where required separating and isolating a desired dihydrotetrabenazine isomer form.

The hydration of the 2,3-double bond can be carried out by hydroboration using a borane reagent such as diborane or a borane-ether (e.g. borane-tetrahydrofuran (THF)) to give an intermediate alkyl borane adduct followed by oxidation of the alkyl borane adduct and hydrolysis in the presence of a base. The hydroboration is typically carried out in a dry polar non-protic solvent such as an ether (e.g. THF), usually at a non-elevated temperature, for example room temperature. The borane-alkene adduct is typically oxidised with an oxidising agent such as hydrogen peroxide in the presence of a base providing a source of hydroxide ions, such as ammonium hydroxide or an alkali metal hydroxide, e.g. potassium hydroxide or sodium hydroxide. The hydroboration-oxidation-hydrolysis sequence of reactions of Process A typically provides dihydrotetrabenazine isomers in which the hydrogen atoms at the 2- and 3-positions have a *trans* relative orientation.

Compounds of the formula (II) can be prepared by reduction of tetrabenazine to give a dihydrotetrabenazine followed by dehydration of the dihydrotetrabenazine. Reduction of the tetrabenazine can be accomplished using an aluminium hydride reagent such as lithium aluminium hydride, or a borohydride reagent such as

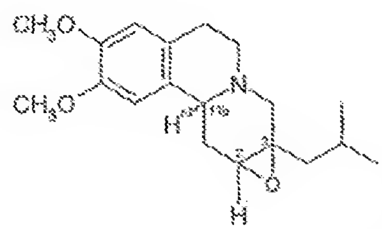
sodium borohydride, potassium borohydride or a borohydride derivative, for example an alkyl borohydride such as lithium tri-*sec*-butyl borohydride. Alternatively, the reduction step can be effected using catalytic hydrogenation, for example over a Raney nickel or platinum oxide catalyst. Suitable conditions for performing the reduction step are described in more detail below or can be found in US 2,843,591 (Hoffmann- La Roche) and Brossi *et al., Helv. Chim. Acta.*, vol. XLI, No. 193, pp1793-1806 (1958).

Because the tetrabenazine used as the starting material for the reduction reaction is typically a mixture of the *RR* and *SS* isomers (*i.e. trans*-tetrabenazine), the dihydrotetrabenazine formed by the reduction step will have the same *trans* configuration about the 3- and 11b positions and will take the form of one or more of the known dihydrotetrabenazine isomers shown in Figure 3 above. Thus Process A may involve taking the known isomers of dihydrotetrabenazine, dehydrating them to form the alkene (II) and then "rehydrating" the alkene (II) using conditions that give the required novel *cis* dihydrotetrabenazine isomers of the invention.

Dehydration of the dihydrotetrabenazine to the alkene (II) can be carried out using a variety of standard conditions for dehydrating alcohols to form alkenes, see for example J. March (*idem*) pages 389-390 and references therein. Examples of such conditions include the use of phosphorus-based dehydrating agents such as phosphorus halides or phosphorus oxyhalides, e.g. $POCl_3$ and PCl_5. As an alternative to direct dehydration, the hydroxyl group of the dihydrotetrabenazine can be converted to a leaving group L such as halogen (e.g. chlorine or bromine) and then subjected to conditions (e.g. the presence of a base) for eliminating H-L. Conversion of the hydroxyl group to a halide can be achieved using methods well known to the skilled chemist, for example by reaction with carbon tetrachloride or carbon tetrabromide in the presence of a trialkyl or triaryl phosphine such as triphenyl phospbine or tributyl phosphine.

The tetrabenazine used as the starting material for the reduction to give the dihydrotetrabenazine can be obtained commercially or can be synthesised by the method described in US 2,830,993 (Hoffmann-La Roche).

Another process (Process B) for preparing a dihydrotetrabenazine of the invention comprises subjecting a compound of the formula (III):



(III)

to conditions for ring-opening the 2,3-epoxide group in the compound of the formula (III), and thereafter where required separating and isolating a desired dihydrotetrabenazine isomer form.

The ring-opening can be effected in accordance with known methods for epoxide ring openings. However, a currently preferred method of ring-opening the epoxide is reductive ring opening which can be achieved using a reducing agent such as borane-THF. Reaction with borane-THF can be carried out in a polar non-protic solvent such as ether (e.g. tetrahydrofuran) usually at ambient temperature, the borane complex thus formed being subsequently hydrolysed by heating in the presence of water and a base at the reflux temperature of the solvent. Process B typically gives rise to dihydrotetrabenazine isomers in which the hydrogen atoms at the 2- and 3-positions have a *cis* relative orientation.

The epoxide compounds of the formula (III) can be prepared by epoxidation of an alkene of the formula (II) above. The epoxidation reaction can be carried out using conditions and reagents well known to the skilled chemist, see for example J. March (*idem*), pages 826-829 and references therein. Typically, a per-acid such as *meta*-chloroperbenzoic acid (MCPBA), or a mixture of a per-acid and a further oxidising agent such as perchloric acid, may be used to bring about epoxidation.

When the starting materials for processes A and B above are mixtures of enantiomers, then the products of the processes will typically be pairs of enantiomers, for example racemic mixtures, possibly together with diastereoisomeric impurities. Unwanted diastereoisomers can be removed by techniques such as chromatography (e.g. HPLC) and the individual enantiomers can

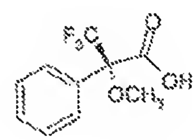
be separated by a variety of methods known to the skilled chemist. For example, they can be separated by means of:

(i) chiral chromatography (chromatography on a chiral support); or

(ii) forming a salt with an optically pure chiral acid, separating the salts of the two diastereoisomers by fractional crystallisation and then releasing the dihydrotetrabenazine from the salt; or

(iii) forming a derivative (such as an ester) with an optically pure chiral derivatising agent (e.g. esterifying agent), separating the resulting epimers (e.g. by chromatography) and then converting the derivative to the dihydrotetrabenazine.

One method of separating pairs of enantiomers obtained from each of Processes A and B, and which has been found to be particularly effective, is to esterify the hydroxyl group of the dihydrotetrabenazine with an optically active form of Mosher's acid, such as the *R* (+) isomer shown below, or an active form thereof:

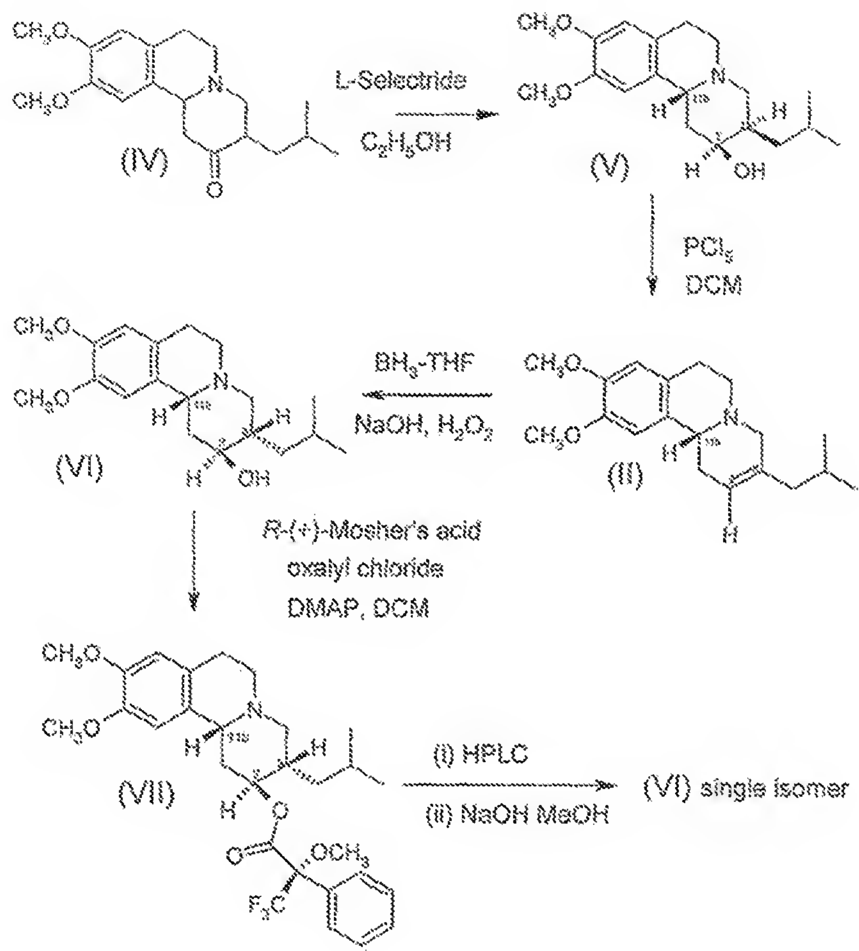


The resulting esters of the two enantiomers of the dihydrobenazine can then be separated by chromatography (e.g. HPLC) and the separated esters hydrolysed to give the individual dihydrobenazine isomers using a base such as an alkali metal hydroxide (e.g. NaOH) in a polar solvent such as methanol.

As an alternative to using mixtures of enantiomers as the starting materials in processes A and B and then carrying out separation of enantiomers subsequently, processes A and B can each be carried out on single enantiomer starting materials leading to products in which a single enantiomer predominates. Single enantiomers of the alkene (II) can be prepared by subjecting RR/SS tetrabenazine to a stereoselective reduction using lithium tri-*sec*-butyl borohydride to give a mixture of SRR and RSS enantiomers of dihydrotetrabenazine, separating the enantiomers (e.g. by fractional crystallisation) and then dehydrating a separated single enantiomer of dihydrotetrabenazine to give predominantly or exclusively a single enantiomer of the compound of formula (II).

Processes A and B are illustrated in more detail below in Schemes 1 and 2 respectively.

Scheme 1



Scheme 1 illustrates the preparation of individual dihydrotetrabenazine isomers having the 2*S*,3*S*,11b*R* and 2*R*,3*R*,11b*S* configurations in which the hydrogen atoms attached to the 2- and 3-positions are arranged in a *trans* relative orientation. This reaction scheme includes Process A defined above.

The starting point for the sequence of reactions in Scheme 1 is commercially available tetrabenazine (IV) which is a racemic mixture of the RR and SS optical isomers of tetrabenazine. In each of the RR and SS isomers, the hydrogen atoms at the 3- and 11b-positions are arranged in a *trans* relative orientation. As an alternative to using the commercially available compound, tetrabenazine can be

synthesised according to the procedure described in US patent number 2,830,993 (see in particular example 11).

The racemic mixture of RR and SS tetrabenazine is reduced using the borohydride reducing agent lithium tri-sec-butyl borohydride ("L-Selectride") to give a mixture of the known 2*S*,3*R*,11b*R* and 2*R*,3*S*,11b*S* isomers (V) of dihydrotetrabenazine, of which only the 2*S*,3*R*,11b*R* isomer is shown for simplicity. By using the more sterically demanding L-Selectride as the borohydride reducing agent rather than sodium borohydride, formation of the RRR and SSS isomers of dihydro-tetrabenazine is minimised or suppressed.

The dihydrotetrabenazine isomers (V) are reacted with a dehydrating agent such as phosphorus pentachloride in a non-protic solvent such as a chlorinated hydrocarbon (for example chloroform or dichloromethane, preferably dichloromethane) to form the unsaturated compound (II) as a pair of enantiomers, only the *R*-enantiomer of which is shown in the Scheme. The dehydration reaction is typically carried out at a temperature lower than room temperature, for example at around 0-5°C.

The unsaturated compound (II) is then subjected to a stereoselective re-hydration to generate the dihydrotetrabenazine (VI) and its mirror image or antipode (not shown) in which the hydrogen atoms at the 3- and 11b-positions are arranged in a *cis* relative orientation and the hydrogen atoms at the 2- and 3-positions are arranged in a *trans* relative orientation. The stereoselective rehydration is accomplished by a hydroboration procedure using borane-THF in tetrahydrofuran (THF) to form an intermediate borane complex (not shown) which is then oxidised with hydrogen peroxide in the presence of a base such as sodium hydroxide.

An initial purification step may then be carried out (e.g. by HPLC) to give the product (V) of the rehydration reaction sequence as a mixture of the 2*S*,3*S*,11b*R* and 2*R*,3*R*,11b*S* isomers of which only the 2*S*,3*S*,11b*R* isomer is shown in the Scheme. In order to separate the isomers, the mixture is treated with *R* (+) Mosher's acid, in the presence of oxalyl chloride and dimethylaminopyridine (DMAP) in dichloromethane to give a pair of diastereoisomeric esters (VII) (of which only one diastereoisomer is shown) which can then be separated using

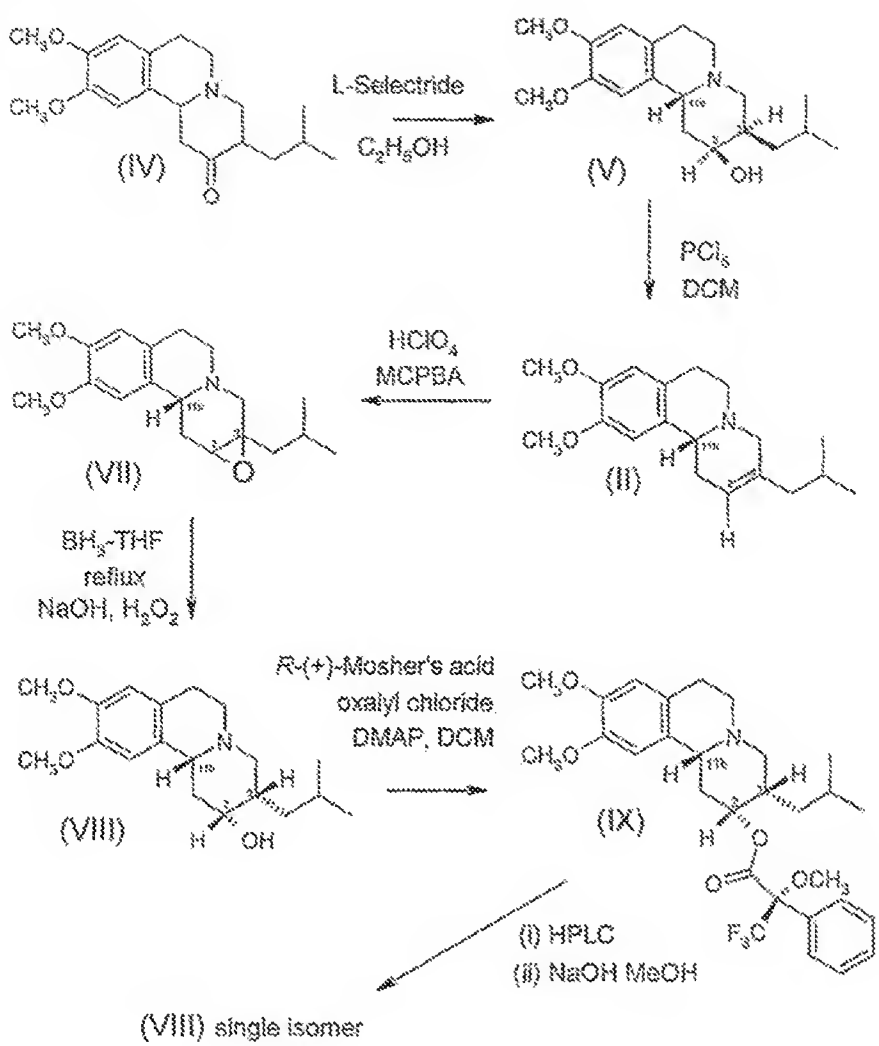
HPLC. The individual esters can then be hydrolysed using an alkali metal hydroxide such as sodium hydroxide to give a single isomer (VI).

In a variation of the sequence of steps shown in Scheme 1, following the reduction of RR/SS tetrabenazine, the resulting mixture of enantiomers of the dihydrotetrabenazine (V) can be separated to give the individual enantiomers. Separation can be carried out by forming a salt with a chiral acid such as (+) or (-) camphorsulphonic acid, separating the resulting diastereoisomers by fractional crystallisation to give a salt of a single enantiomer and then releasing the free base from the salt.

The separated dihydrotetrabenazine enantiomer can be dehydrated to give a single enantiomer of the alkene (II). Subsequent rehydration of the alkene (II) will then give predominantly or exclusively a single enantiomer of the cis-dihydrotetrabenazine (VI). An advantage of this variation is that it does not involve the formation of Mosher's acid esters and therefore avoids the chromatographic separation typically used to separate Mosher's acid esters.

Scheme 2 illustrates the preparation of individual dihydrotetrabenazine isomers having the 2*R*,3*S*,11b*R* and 2*S*,3*R*,11b*S* configurations in which the hydrogen atoms attached to the 2- and 3-positions are arranged in a *cis* relative orientation. This reaction scheme includes Process B defined above.

Scheme 2



In Scheme 2, the unsaturated compound (II) is produced by reducing tetrabenazine to give the 2S,3R,11bR and 2R,3S,11bS isomers (V) of dihydrotetrabenazine and dehydrating with PCl_5 in the manner described above in Scheme 1. However, instead of subjecting the compound (II) to hydroboration, the 2,3-double bond is converted to an epoxide by reaction with *meta*-chloroperbenzoic acid (MCPBA) and perchloric acid. The epoxidation reaction is conveniently carried out in an alcohol solvent such as methanol, typically at around room temperature.

The epoxide (VII) is then subjected to a reductive ring opening using borane-THF as an electrophilic reducing agent to give an intermediate borane complex (not shown) which is then oxidised and cleaved with hydrogen peroxide in the presence

of an alkali such as sodium hydroxide to give a dihydrotetrabenazine (VIII) as a mixture of the 2*R*,3*S*,11b*R* and 2*S*,3*R*,11b*S* isomers, of which only the 2*R*,3*S*,11b*R* is shown for simplicity. Treatment of the mixture of isomers (VIII) with *R* (+) Mosher's acid in the presence of oxalyl chloride and dimethylaminopyridine (DMAP) in dichloromethane gives a pair of epimeric esters (IX) (of which only one epimer is shown) which can then by separated by chromatography and hydrolysed with sodium hydroxide in methanol in the manner described above in relation to Scheme 1.

Pharmaceutical Formulations

The *cis*-dihydrotetrabenazine compounds of the invention are typically administered in the form of pharmaceutical compositions.

The pharmaceutical compositions can be in any form suitable for oral, parenteral, topical, intranasal, intrabronchial, ophthalmic, otic, rectal, intra-vaginal, or transdermal administration. Where the compositions are intended for parenteral administration, they can be formulated for intravenous, intramuscular, intraperitoneal, subcutaneous administration or for direct delivery into a target organ or tissue by injection, infusion or other means of delivery.

Pharmaceutical dosage forms suitable for oral administration include tablets, capsules, caplets, pills, lozenges, syrups, solutions, sprays, powders, granules, elixirs and suspensions, sublingual tablets, sprays, wafers or patches and buccal patches.

Pharmaceutical compositions containing the dihydrotetrabenazine compounds of the invention can be formulated in accordance with known techniques, see for example, Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, PA, USA.

Thus, tablet compositions can contain a unit dosage of active compound together with an inert diluent or carrier such as a sugar or sugar alcohol, e.g.; lactose, sucrose, sorbitol or mannitol; and/or a non-sugar derived diluent such as sodium carbonate, calcium phosphate, talc, calcium carbonate, or a cellulose or derivative

thereof such as methyl cellulose, ethyl cellulose, hydroxypropyl methyl cellulose, and starches such as corn starch. Tablets may also contain such standard ingredients as binding and granulating agents such as polyvinylpyrrolidone, disintegrants (e.g. swellable crosslinked polymers such as crosslinked carboxymethylcellulose), lubricating agents (e.g. stearates), preservatives (e.g. parabens), antioxidants (e.g. BHT), buffering agents (for example phosphate or citrate buffers), and effervescent agents such as citrate/bicarbonate mixtures. Such excipients are well known and do not need to be discussed in detail here.

Capsule formulations may be of the hard gelatin or soft gelatin variety and can contain the active component in solid, semi-solid, or liquid form. Gelatin capsules can be formed from animal gelatin or synthetic or plant derived equivalents thereof.

The solid dosage forms (e.g.; tablets, capsules etc.) can be coated or un-coated, but typically have a coating, for example a protective film coating (e.g. a wax or varnish) or a release controlling coating. The coating (e.g. a Eudragit ™ type polymer) can be designed to release the active component at a desired location within the gastro-intestinal tract. Thus, the coating can be selected so as to degrade under certain pH conditions within the gastrointestinal tract, thereby selectively release the compound in the stomach or in the ileum or duodenum.

Instead of, or in addition to, a coating, the drug can be presented in a solid matrix comprising a release controlling agent, for example a release delaying agent which may be adapted to selectively release the compound under conditions of varying acidity or alkalinity in the gastrointestinal tract. Alternatively, the matrix material or release retarding coating can take the form of an erodible polymer (e.g. a maleic anhydride polymer) which is substantially continuously eroded as the dosage form passes through the gastrointestinal tract.

Compositions for topical use include ointments, creams, sprays, patches, gels, liquid drops and inserts (for example intraocular inserts). Such compositions can be formulated in accordance with known methods.

Compositions for parenteral administration are typically presented as sterile aqueous or oily solutions or fine suspensions, or may be provided in finely divided

sterile powder form for making up extemporaneously with sterile water for injection.

Examples of formulations for rectal or intra-vaginal administration include pessaries and suppositories which may be, for example, formed from a shaped mouldable or waxy material containing the active compound.

Compositions for administration by inhalation may take the form of inhalable powder compositions or liquid or powder sprays, and can be administrated in standard form using powder inhaler devices or aerosol dispensing devices. Such devices are well known. For administration by inhalation, the powdered formulations typically comprise the active compound together with an inert solid powdered diluent such as lactose.

The compounds of the inventions will generally be presented in unit dosage form and, as such, will typically contain sufficient compound to provide a desired level of biological activity. For example, a formulation intended for oral administration may contain from 2 milligrams to 200 milligrams of active ingredient, more usually from 10 milligrams to 100 milligrams, for example, 12.5 milligrams, 25 milligrams and 50 milligrams.

Methods of Treatment

The active compound will be administered to a patient in need thereof (for example a human or animal patient) in an amount sufficient to achieve the desired therapeutic effect.

The patient in need of such administration is a patient suffering from or exhibiting, or at risk of suffering from or exhibiting, one or more psychoses, for example a psychosis characteristic of schizophrenia.

The desired effect can be the prevention, alleviation or reduction of the severity of the psychosis or one or more symptoms thereof. Such symptoms are well known to the skilled person (e.g. a skilled physician) who will be able to judge through clinical evaluation and testing in a conventional manner whether or not the

administration of a compound of the invention has resulted in a change in the symptoms exhibited by the patient.

The compounds will typically be administered in amounts that are therapeutically or prophylactically useful and which generally are non-toxic. However, in certain situations, the benefits of administering a dihydrotetrabenazine compound of the invention may outweigh the disadvantages of any toxic effects or side effects, in which case it may be considered desirable to administer compounds in amounts that are associated with a degree of toxicity.

A typical daily dose of the compound can be up to 1000 mg per day, for example in the range from 0.01 milligrams to 10 milligrams per kilogram of body weight, more usually from 0.025 milligrams to 5 milligrams per kilogram of body weight, for example up to 3 milligrams per kilogram of bodyweight, and more typically 0.15 milligrams to 5 milligrams per kilogram of bodyweight although higher or lower doses may be administered where required.

Ultimately, however, the quantity of compound administered will be commensurate with the nature of the disease or physiological condition being treated and the therapeutic benefits and the presence or absence of side effects produced by a given dosage regimen, and will be at the discretion of the physician.

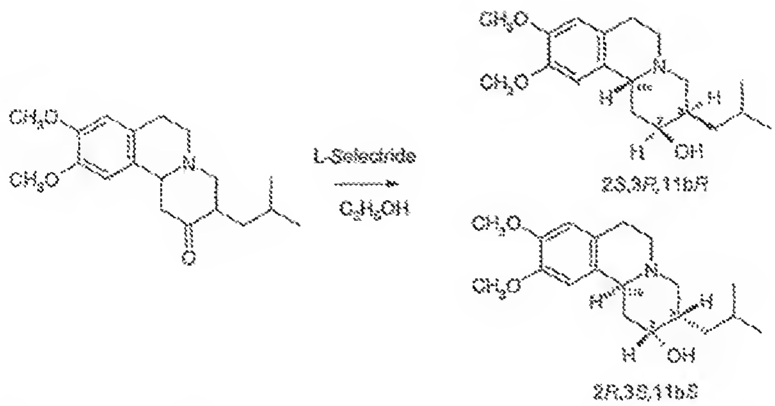
EXAMPLES

The following non-limiting examples illustrate the synthesis and properties of the dihydrotetrabenazine compounds of the invention.

EXAMPLE 1

Preparation of 2*S*,3*S*,11b*R* and 2*R*,3*R*,11b*S* Isomers of Dihydrotetrabenazine

1A. Reduction of *RR*/*SS* Tetrabenazine

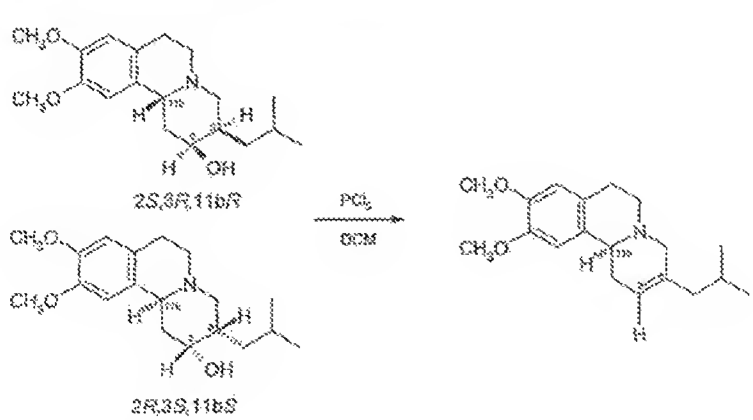


1M L-Selectride® in tetrahydrofuran (135 ml, 135 mmol, 2.87 eq.) was added slowly over 30 minutes to a stirred solution of tetrabenazine *RR/SS* racemate (15 g, 47 mmol) in ethanol (75 ml) and tetrahydrofuran (75 ml) at 0 °C. After addition was complete the mixture was stirred at 0 °C for 30 minutes and then allowed to warm to room temperature.

The mixture was poured onto crushed ice (300 g) and water (100 ml) added. The solution was extracted with diethyl ether (2 x 200 ml) and the combined ethereal extracts washed with water (100 ml) and partly dried over anhydrous potassium carbonate. Drying was completed using anhydrous magnesium sulphate and, after filtration, the solvent was removed at reduced pressure (shielded from the light, bath temperature <20 °C) to afford a pale yellow solid.

The solid was slurried with petroleum ether (30-40 °C) and filtered to afford a white powdery solid (12 g, 80%).

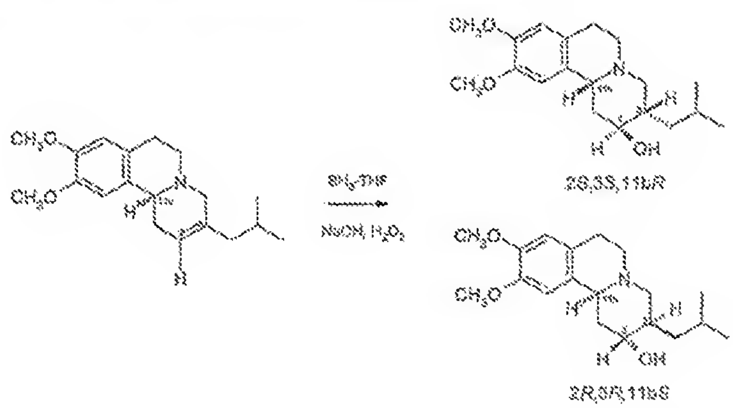
<u>1B. Dehydration of reduced Tetrabenazine</u>



Phosphorous pentachloride (32.8 g, 157.5 mmol, 2.5 eq) was added in portions over 30 minutes to a stirred solution of the reduced tetrabenazine product from Example 1A (20 g, 62.7 mmol) in dichloromethane (200 ml) at 0 °C. After the addition was complete, the reaction mixture was stirred at 0 °C for a further 30 minutes and the solution poured slowly into 2M aqueous sodium carbonate solution containing crushed ice (0 °C). Once the initial acid gas evolution had ceased the mixture was basified (ca. pH 12) using solid sodium carbonate.

The alkaline solution was extracted using ethyl acetate (800 ml) and the combined organic extracts dried over anhydrous magnesium sulphate. After filtration the solvent was removed at reduced pressure to afford a brown oil, which was purified by column chromatography (silica, ethyl acetate) to afford the semi-pure alkene as a yellow solid (10.87 g, 58%).

1C. Hydration of the Crude Alkene from Example 1B



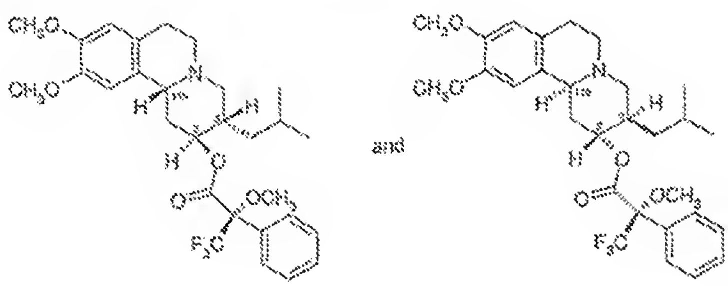
A solution of the crude alkene (10.87 g, 36.11 mmol) from Example 1B in dry THF (52 ml) at room temperature was treated with 1M borane-THF (155.6 ml, 155.6 mmol, 4.30 eq) added in a dropwise manner. The reaction was stirred for 2 hours, water (20 ml) was added and the solution basified to pH 12 with 30% aqueous sodium hydroxide solution.

Aqueous 30% hydrogen peroxide solution (30 ml) was added to the stirred alkaline reaction mixture and the solution was heated to reflux for 1 hour before being allowed to cool. Water (100 ml) was added and the mixture extracted with ethyl acetate (3 x 250 ml). The organic extracts were combined and dried over

anhydrous magnesium sulphate and after filtration the solvent was removed at reduced pressure to afford a yellow oil (9 g).

The oil was purified using preparative HPLC (Column: Lichrospher Si60, 5 µm, 250 x 21.20 mm, mobile phase: hexane : ethanol : dichloromethane (85:15:5); UV 254 nm, flow: 10 ml min^{-1}) at 350 mg per injection followed by concentration of the fractions of interest under vacuum. The product oil was then dissolved in ether and concentrated once more under vacuum to give the dihydrotetrabenazine racemate shown above as a yellow foam (5.76 g, 50%).

1D. Preparation of Mosher's ester derivatives



R-(+)-α-methoxy-α-trifluoromethylphenyl acetic acid (5 g, 21.35 mmol), oxalyl chloride (2.02 ml) and DMF (0.16 ml) were added to anhydrous dichloromethane (50 ml) and the solution was stirred at room temperature for 45 minutes. The solution was concentrated under reduced pressure and the residue was taken up in anhydrous dichloromethane (50 ml) once more. The resulting solution was cooled using an ice-water bath and dimethylaminopyridine (3.83 g, 31.34 mmol) was added followed by a pre-dried solution (over 4Å sieves) in anhydrous dichloromethane of the solid product of Example 1C (5 g, 15.6 mmol). After stirring at room temperature for 45 minutes, water (234 ml) was added and the mixture extracted with ether (2 x 200 ml). The ether extract was dried over anhydrous magnesium sulphate, passed through a pad of silica and the product eluted using ether.

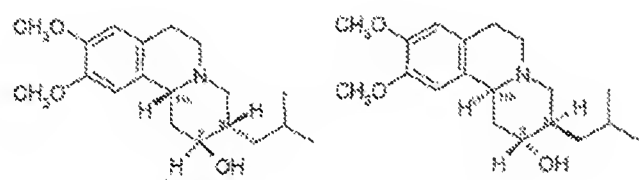
The collected ether eluate was concentrated under reduced pressure to afford an oil which was purified using column chromatography (silica, hexane : ether (10:1)). Evaporation of the collected column fractions of interest and removal of the solvent

at reduced pressure gave a solid which was further purified using column chromatography (silica, hexane : ethyl acetate (1:1)) to give three main components which were partially resolved into Mosher's ester peaks 1 and 2.

Preparative HPLC of the three components (Column: 2 x Lichrospher Si60, 5 μm, 250 x 21.20 mm, mobile phase: hexane : isopropanol (97:3), UV 254 nm; flow: 10 ml min^{-1}) at 300 mg loading followed by concentration of the fractions of interest under vacuum gave the pure Mosher's ester derivatives

Peak 1 (3.89 g, 46.5%)
Peak 2 (2.78 g, 33%)

The fractions corresponding to the two peaks were subjected to hydrolysis to liberate the individual dihydrotetrabenazine isomers identified and characterised as Isomers A and B. Isomers A and B are each believed to have one of the following structures



2S,3S,11bR 2R,3R,11bS

More specifically, Isomer B is believed to have the 2*S*, 3*S*, 11b*R* absolute configuration on the basis of the X-ray crystallography experiments described in Example 4 below.

1E. Hydrolysis of Peak 1 to give Isomer A

Aqueous 20% sodium hydroxide solution (87.5 ml) was added to a solution of Mosher's ester peak 1 (3.89 g, 7.27 mmol) in methanol (260 ml) and the mixture stirred and heated to reflux for 150 minutes. After cooling to room temperature water (200 ml) was added and the solution extracted with ether (600 ml), dried over anhydrous magnesium sulphate and after filtration, concentrated under reduced pressure.

The residue was dissolved using ethyl acetate (200 ml), the solution washed with water (2 x 50 ml), the organic phase dried over anhydrous magnesium sulphate and after filtration, concentrated under reduced pressure to give a yellow foam. This material was purified by column chromatography (silica, gradient elution of ethyl acetate : hexane (1:1) to ethyl acetate). The fractions of interest were combined and the solvent removed at reduced pressure. The residue was taken up in ether and the solvent removed at reduced pressure once more to give Isomer A as an off-white foam (1.1 g, 47%).

Isomer A, which is believed to have the 2*R*,3*R*,11b*S* configuration (the absolute stereochemistry was not determined), was characterized by ^{1}H-NMR, ^{13}C-NMR, IR, mass spectrometry, chiral HPLC and ORD. The IR, NMR and MS data for isomer A are set out in Table 1 and the Chiral HPLC and ORD data are set out in Table 3.

1F. Hydrolysis of Peak 2 to give Isomer B

Aqueous 20% sodium hydroxide solution (62.5 ml) was added to a solution of Mosher's ester peak 2 (2.78 g, 5.19 mmol) in methanol (185 ml) and the mixture stirred and heated to reflux for 150 minutes. After cooling to room temperature water (142 ml) was added and the solution extracted with ether (440 ml), dried over anhydrous magnesium sulphate and after filtration, concentrated under reduced pressure.

The residue was dissolved using ethyl acetate (200 ml), the solution washed with water (2 x 50 ml), the organic phase dried over anhydrous magnesium sulphate and after filtration, concentrated under reduced pressure. Petroleum ether (30-40 °C) was added to the residue and the solution concentrated under vacuum once more to give Isomer B as a white foam (1.34 g, 81%).

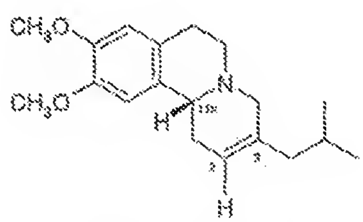
Isomer B, which is believed to have the 2*S*,3*S*,11b*R* configuration, was characterized by ^{1}H-NMR, ^{13}C-NMR, IR, mass spectrometry, chiral HPLC, ORD and X-ray crystallography. The IR, NMR and MS data for Isomer B are set out in Table 1 and the Chiral HPLC and ORD data are set out in Table 3. The X-ray crystallography data are set out in Example 4.

EXAMPLE 2

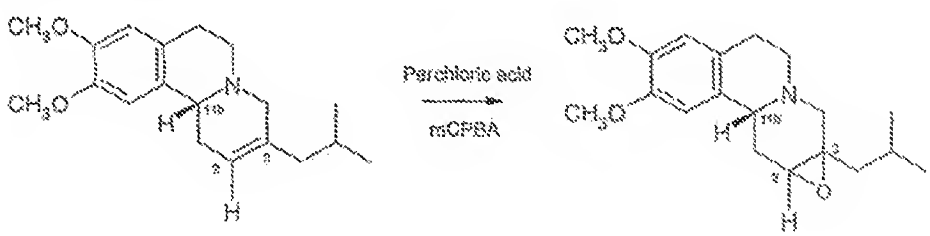
Preparation of 2*R*,3*S*,11b*R* and 2*S*,3*R*,11b*S* Isomers of Dihydrotetrabenazine

2A. Preparation of 2,3-Dehydrotetrabenazine

A solution containing a racemic mixture (15 g, 47 mmol) of *RR* and *SS* tetrabenazine enantiomers in tetrahydrofuran was subjected to reduction with L-Selectride® by the method of Example 1A to give a mixture of the 2*S*,3*R*,11b*R* and 2*R*,3*S*,11b*S* enantiomers of dihydrotetrabenazine.as a white powdery solid (12 g, 80%). The partially purified dihydrotetrabenazine was then dehydrated using PCl_5 according to the method of Example 1B to give a semi-pure mixture of 11b*R* and 11b*S* isomers of 2,3-dehydrotetrabenazine (the 11b*R* enantiomer of which is shown below) as a yellow solid (12.92 g, 68%).



2B. Epoxidation of the Crude Alkene from Example 2A



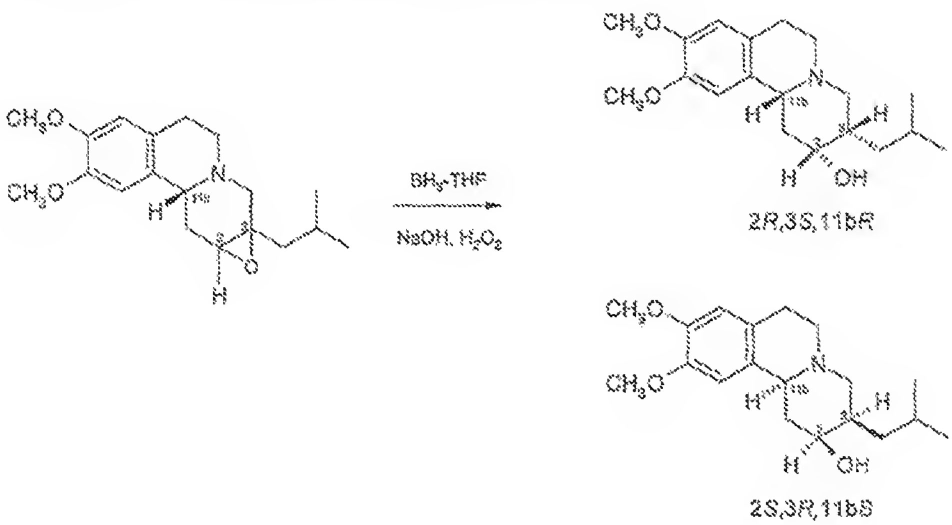
To a stirred solution of the crude alkene from Example 2A (12,92 g, 42.9 mmol) in methanol (215 ml) was added a solution of 70% perchloric acid (3.70 ml, 43 mmol) in methanol (215 ml). 77% 3-Chloroperoxybenzoic acid (15.50 g, 65 mmol) was added to the reaction and the resulting mixture was stirred for 18 hours at room temperature protected from light.

The reaction mixture was poured into saturated aqueous sodium sulphite solution (200 ml) and water (200 ml) added. Chloroform (300 ml) was added to the

resulting emulsion and the mixture basified with saturated aqueous sodium bicarbonate (400 ml).

The organic layer was collected and the aqueous phase washed with additional chloroform (2 x 150 ml). The combined chloroform layers were dried over anhydrous magnesium sulphate and after filtration the solvent was removed at reduced pressure to give a brown oil (14.35 g, yield > 100% - probable solvent remains in product). This material was used without further purification.

2C. Reductive Ring Opening of the Epoxide from 2B



A stirred solution of the crude epoxide from Example 2B (14.35 g, 42.9 mmol, assuming 100% yield) in dry THF (80 ml) was treated slowly with 1M borane/THF (184.6 ml, 184.6 mmol) over 15 minutes. The reaction was stirred for two hours, water (65 ml) was added and the solution heated with stirring to reflux for 30 minutes.

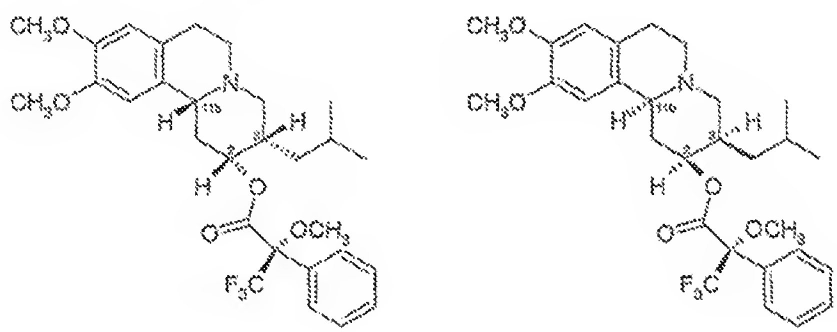
After cooling, 30% sodium hydroxide solution (97 ml) was added to the reaction mixture followed by 30% hydrogen peroxide solution (48.6 ml) and the reaction was stirred and heated to reflux for an additional 1 hour.

The cooled reaction mixture was extracted with ethyl acetate (500 ml) dried over anhydrous magnesium sulphate and after filtration the solvent was removed at

reduced pressure to give an oil. Hexane (230 ml) was added to the oil and the solution re-concentrated under reduced pressure.

The oily residue was purified by column chromatography (silica, ethyl acetate). The fractions of interest were combined and the solvent removed under reduced pressure. The residue was purified once more using column chromatography (silica, gradient, hexane to ether). The fractions of interest were combined and the solvents evaporated at reduced pressure to give a pale yellow solid (5.18 g, 38%).

<u>2D. Preparation of Mosher's ester derivatives of the 2*R*,3*S*,11b*R* and 2*S*,3*R*,11b*S* Isomers of Dihydrotetrabenazine</u>



R-(+)-α-methoxy-α-trifluoromethylphenyl acetic acid (4.68 g, 19.98 mmol), oxalyl chloride (1.90 ml) and DMF (0.13 ml) were added to anhydrous dichloromethane (46 ml) and the solution stirred at room temperature for 45 minutes. The solution was concentrated under reduced pressure and the residue was taken up in anhydrous dichloromethane (40 ml) once more. The resulting solution was cooled using an ice-water bath and dimethylaminopyridine (3.65 g, 29.87 mmol) was added followed by a pre-dried solution (over 4Å sieves) in anhydrous dichloromethane (20 ml) of the solid product of Example 2C (4.68 g, 14.6 mmol). After stirring at room temperature for 45 minutes, water (234 ml) was added and the mixture extracted with ether (2 x 200 ml). The ether extract was dried over anhydrous magnesium sulphate, passed through a pad of silica and the product eluted using ether.

The collected ether eluate was concentrated under reduced pressure to afford an oil which was purified using column chromatography (silica, hexane : ether (1:1)).

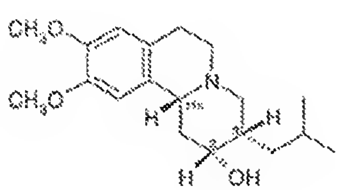
Evaporation of the collected column fractions of interest and removal of the solvent at reduced pressure gave a pink solid (6.53 g)

Preparative HPLC of the solid (Column: 2 x Lichrospher Si60, 5 μm, 250 x 21.20 mm; mobile phase hexane : isopropanol (97:3); UV 254 nm; flow: 10 ml min^{-1}) at 100 mg loading followed by concentration of the fractions of interest under vacuum gave a solid which was slurried with petroleum ether (30-40 °C) and collected by filtration to give the pure Mosher's ester derivatives

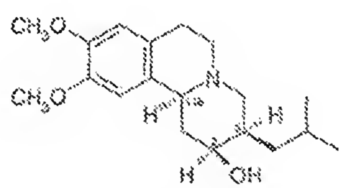
Peak 1 (2.37 g, 30%)

Peak 2 (2.42 g, 30%)

The fractions corresponding to the two peaks were subjected to hydrolysis to liberate the individual dihydrotetrabenazine isomers identified and characterised as Isomers C and D. Isomers C and D are each believed to have one of the following structures



2R,3S,11bR



2S,3R,11bS

2F. Hydrolysis of Peak 1 to give Isomer C

20% aqueous sodium hydroxide solution (53 ml) was added to a stirred solution of Mosher's ester peak 1 (2.37 g, 4.43 mmol) in methanol (158 ml) and the mixture stirred at reflux for 150 minutes. After cooling water (88 ml) was added to the reaction mixture and the resulting solution extracted with ether (576 ml). The organic extract was dried over anhydrous magnesium sulphate and after filtration the solvent removed at reduced pressure. Ethyl acetate (200 ml) was added to the residue and the solution washed with water (2 x 50 ml). The organic solution was dried over anhydrous magnesium sulphate and after filtration the solvent removed at reduced pressure.

This residue was treated with petroleum ether (30-40 °C) and the resulting suspended solid collected by filtration. The filtrate was concentrated at reduced pressure and the second batch of suspended solid was collected by filtration. Both collected solids were combined and dried under reduced pressure to give Isomer C (1.0 g, 70%).

Isomer C, which is believed to have either the 2*R*,3*S*,11b*R* or 2*S*,3*R*,11b*S* configuration (the absolute stereochemistry was not determined), was characterized by ^{1}H-NMR, ^{13}C-NMR, IR, mass spectrometry, chiral HPLC and ORD. The IR, NMR and MS data for Isomer C are set out in Table 2 and the Chiral HPLC and ORD data are set out in Table 4.

2G. Hydrolysis of Peak 2 to give Isomer D

20% aqueous sodium hydroxide solution (53 ml) was added to a stirred solution of Mosher's ester peak 2 (2.42 g, 4.52 mmol) in methanol (158 ml) and the mixture stirred at reflux for 150 minutes. After cooling water (88 ml) was added to the reaction mixture and the resulting solution extracted with ether (576 ml). The organic extract was dried over anhydrous magnesium sulphate and after filtration the solvent removed at reduced pressure. Ethyl acetate (200 ml) was added to the residue and the solution washed with water (2 x 50 ml). The organic solution was dried over anhydrous magnesium sulphate and after filtration the solvent removed at reduced pressure.

This residue was treated with petroleum ether (30-40 °C) and the resulting suspended orange solid collected by filtration. The solid was dissolved in ethyl acetate : hexane (15:85) and purified by column chromatography (silica, gradient ethyl acetate : hexane (15:85) to ethyl acetate). The fractions of interest were combined and the solvent removed at reduced pressure. The residue was slurried with petroleum ether (30-40 °C) and the resulting suspension collected by filtration. The collected solid was dried under reduced pressure to give Isomer D as a white solid (0.93 g, 64%).

Isomer D, which is believed to have either the 2*R*,3*S*,11b*R* or 2*S*,3*R*,11b*S* configuration (the absolute stereochemistry was not determined), was characterized

by ^{1}H-NMR, ^{13}C-NMR, IR, mass spectrometry, chiral HPLC and ORD. The IR, NMR and MS data for Isomer D are set out in Table 2 and the Chiral HPLC and ORD data are set out in Table 4.

In Tables 1 and 2, the infra red spectra were determined using the KBr disc method. The ^{1}H NMR spectra were carried out on solutions in deuterated chloroform using a Varian Gemini NMR spectrometer (200 MHz.). The ^{13}C NMR spectra were carried out on solutions in deuterated chloroform using a Varian Gemini NMR spectrometer (50MHz). The mass spectra were obtained using a Micromass Platform II (ES^+ conditions) spectrometer. In Tables 3 and 4, the Optical Rotatory Dispersion figures were obtained using an Optical Activity PolAAr 2001 instrument in methanol solution at 24°C. The HPLC retention time measurements were carried out using an HP1050 HPLC chromatograph with UV detection.

<u>Tables 1 and 2 – Spectroscopic Data</u>

Table 1

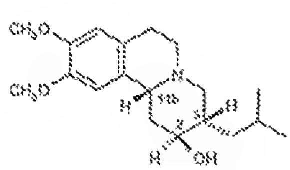
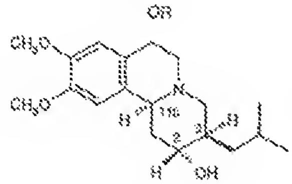
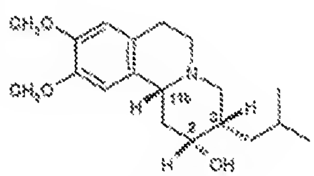
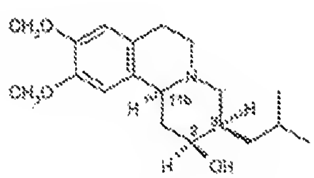
Dihydrotetrabenazine isomer	^{1}H-NMR spectrum ($CDCl_3$)	^{13}C-NMR spectrum ($CDCl_3$)	IR Spectrum (KBr solid)	Mass Spectrum (ES^+)
Isomers A and B  2S,3S,11bR OR  2R,3R,11bS	6.67δ 1H (s); 6.57 δ 1H (s); 3.84 δ 6H (s); 3.55 δ 1H (br. d); 3.08 δ 1H (m); 2.79 δ 2H (m); 2.55 δ 3H (m); 2.17 δ 1H (m); 1.72 δ 6H (m); 1.02 δ 1H (m); 0.88 δ 6H (t)	147.7δ; 147.6δ; 130.5δ; 127.6δ; 112.1δ; 108.4δ; 70.5δ; 57.5 δ; 56.5 δ; 56.3 δ; 54.8 δ; 53.2 δ; 40.4 δ; 40.1 δ; 36.0 δ; 28.8 δ;	2950 cm^{-1}; 2928 cm^{-1}; 2868 cm^{-1}; 2834 cm^{-1}; 1610 cm^{-1}; 1511 cm^{-1}; 1464 cm^{-1} 1364 cm^{-1}; 1324 cm^{-1}; 1258 cm^{-1}; 1223 cm^{-1}; 1208 cm^{-1}; 1144 cm^{-1}; 1045 cm^{-1}; 1006 cm^{-1}; 870 cm^{-1};	MH^+ 320

Table 1				
Dihydrotetrabenazine isomer	^{1}H-NMR spectrum ($CDCl_3$)	^{13}C-NMR spectrum ($CDCl_3$)	IR Spectrum (KBr solid)	Mass Spectrum (ES^+)
		26.2 δ; 23.7δ; 22.9 δ	785 cm^{-1}; 764 cm^{-1}	

Table 2				
Dihydrotetrabenazine isomer	^{1}H-NMR spectrum ($CDCl_3$)	^{13}C-NMR spectrum ($CDCl_3$)	IR Spectrum (KBr solid)	Mass Spectrum (ES^+)
Isomers C and D  2R,3S,11bR OR  2S,3R,11bS	6.68 δ 1H (s); 6.58 δ 1H (s); 3.92 δ 1H (m); 3.84 δ 6H (s); 3.15 δ 1H (m); 2.87 δ 3H (m); 2.43 δ 4H (m); 1.81 δ 1H (m); 1.64 δ 4H (m); 1.21 δ 1H (m); 0.94 δ 3H (d); 0.89 δ 3H (d)	147.8 δ; 147.7 δ; 130.4 δ; 127.2 δ; 112.0 δ; 108.3 δ; 72.4 δ; 61.2 δ; 58.3 δ; 56.5 δ; 56.3 δ; 52.7 δ; 38.6 δ; 36.7 δ; 34.4 δ; 29.6 δ; 26.5 δ; 24.4 δ; 22.5 δ	3370 cm^{-1}; 2950 cm^{-1}; 2929 cm^{-1}; 1611 cm^{-1}; 1512 cm^{-1}; 1463 cm^{-1} 1362 cm^{-1}; 1334 cm^{-1}; 1259 cm^{-1}; 1227 cm^{-1}; 1148 cm^{-1}; 1063 cm^{-1}; 1024 cm^{-1}; 855 cm^{-1}; 766 cm^{-1}	MH^+ 320

<u>Tables 3 and 4 – Chromatography and ORD Data</u>

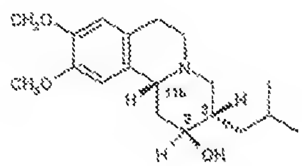
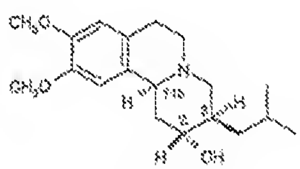
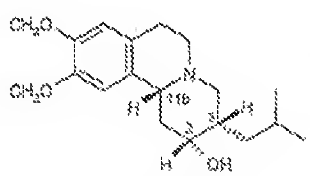
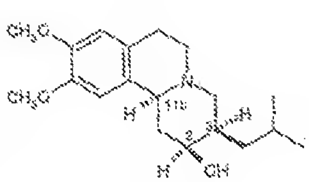
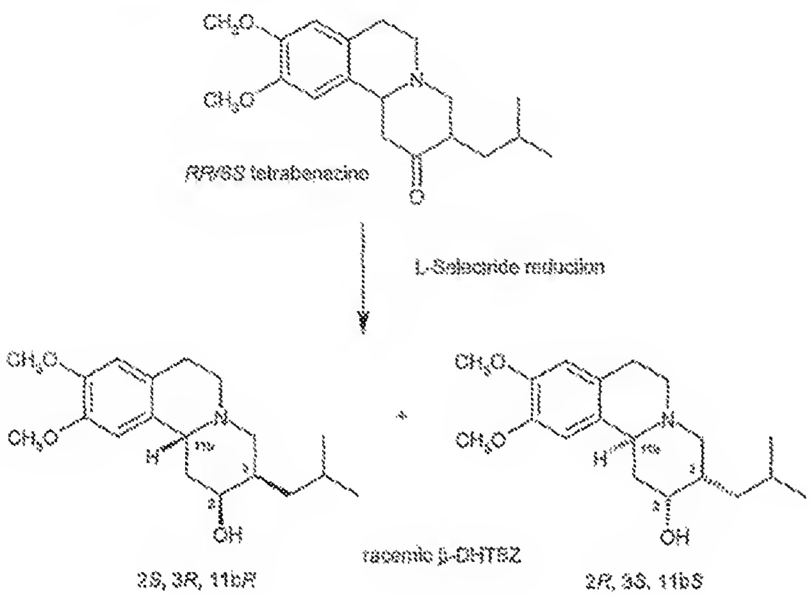
Table 3		
Dihydrotetrabenazine isomer	Chiral HPLC Methods and Retention Times	ORD (MeOH, 21°C)
Isomers A and B  2S,3S,11bR OR  2R,3R,11bS	Column: Chirex (S)-VAL, (R)-NEA, 250 x 4.6 mm Mobile phase: Hexane : 1,2-dichloroethane : ethanol (36:62:2) Flow: 1.0 ml min^{-1} UV: 254 nm Retention times: Isomer A 16.6 min Isomer B 15.3 min	Isomer A $[\alpha_D]$-114.6° Isomer B $[\alpha_D]$ +123°

Table 4		
Isomers C and D  2R,3S,11bR OR  2S,3R,11bS	Column: Chirex (S)-VAL, (R)-NEA, 250 x 4.6mm Mobile phase: Hexane : ethanol (92:8) Flow: 1.0 ml min^{-1} UV: 254 nm Retention times: Isomer C 20.3 min Isomer D 19.4 min	Isomer C $[\alpha_D]$ +150.9° Isomer D $[\alpha_D]$ -145.7°

EXAMPLE 3

Alternative Method of Preparation of Isomer B and Preparation of Mesylate Salt

3A. Reduction of *RR/SS* Tetrabenazine



1M L-Selectride® in tetrahydrofuran (52 ml, 52 mmol, 1.1 eq) was added slowly over 30 minutes to a cooled (ice bath), stirred solution of tetrabenazine racemate (15 g, 47 mmol) in tetrahydrofuran (56 ml). After the addition was complete, the mixture was allowed to warm to room temperature and stirred for a further six hours. TLC analysis (silica, ethyl acetate) showed only very minor amounts of starting material remained.

The mixture was poured on to a stirred mixture of crushed ice (112 g), water (56 ml) and glacial acetic acid (12.2 g). The resulting yellow solution was washed with ether (2 x 50 ml) and basified by the slow addition of solid sodium carbonate (ca. 13 g). Pet-ether (30-40 °C) (56 ml) was added to the mixture with stirring and the crude β-DHTBZ was collected as a white solid by filtration.

The crude solid was dissolved in dichloromethane (ca. 150 ml) and the resulting solution washed with water (40 ml), dried using anhydrous magnesium sulphate, filtered and concentrated at reduced pressure to ca. 40 ml. A thick suspension of white solid was formed. Pet-ether (30-40 °C) (56 ml) was added and the

suspension was stirred for fifteen minutes at laboratory temperature. The product was collected by filtration and washed on the filter until snow-white using pet-ether (30-40°C) (40 to 60 ml) before air-drying at room temperature to yield β-DHTBZ (10.1 g, 67%) as a white solid. TLC analysis (silica, ethyl acetate) showed only one component.

3B. Preparation and Fractional Crystallisation of the Camphorsulphonic acid Salt of Racemic β-DHTBZ

The product of Example 3A and 1 equivalent of (*S*)-(+)-Camphor-10-sulphonic acid were dissolved with heating in the minimum amount of methanol. The resulting solution was allowed to cool and then diluted slowly with ether until formation of the resulting solid precipitation was complete. The resulting white crystalline solid was collected by filtration and washed with ether before drying.

The camphorsulphonic acid salt of (10 g) was dissolved in a mixture of hot absolute ethanol (170 ml) and methanol (30 ml). The resulting solution was stirred and allowed to cool. After two hours the precipitate formed was collected by filtration as a white crystalline solid (2.9 g). A sample of the crystalline material was shaken in a separating funnel with excess saturated aqueous sodium carbonate and dichloromethane. The organic phase was separated, dried over anhydrous magnesium sulphate, filtered and concentrated at reduced pressure. The residue was triturated using pet-ether (30-40 °C) and the organic solution concentrated once more. Chiral HPLC analysis of the salt using a Chirex (S)-VAL and (R)-NEA 250 x 4.6 mm column, and a hexane : ethanol (98:2) eluent at a flow rate of 1 ml/minute showed showed that the isolated β-DHTBZ was enriched in one enantiomer (e.e. ca. 80%).

The enriched camphorsulphonic acid salt (14 g) was dissolved in hot absolute ethanol (140 ml) and propan-2-ol (420 ml) was added. The resulting solution was stirred and a precipitate began to form within one minute. The mixture was allowed to cool to room temperature and stirred for one hour. The precipitate formed was collected by filtration, washed with ether and dried to give a white crystalline solid (12 g).

The crystalline material was shaken in a separating funnel with excess saturated aqueous sodium carbonate and dichloromethane. The organic phase was separated, dried over anhydrous magnesium sulphate, filtered and concentrated at reduced pressure. The residue was triturated using pet-ether (30-40 °C) and the organic solution concentrated once more to yield (after drying in vacuo.) (+)-β-DHTBZ (6.6 g, ORD +107.8°). The isolated enantiomer has e.e. >97%.

3C. Preparation of Isomer B

A solution of phosphorus pentachloride (4.5 g, 21.6 mmol, 1.05 eq) in dichloromethane (55 ml) was added steadily over ten minutes to a stirred, cooled (ice-water bath) solution of the product of Example 3B (6.6 g, 20.6 mmol) in dichloromethane (90 ml). When the addition was complete, the resulting yellow solution was stirred for a further ten minutes before pouring on to a rapidly stirred mixture of sodium carbonate (15 g) in water (90 ml) and crushed ice (90 g). The mixture was stirred for a further 10 minutes and transferred to a separating funnel.

Once the phases had separated, the brown dichloromethane layer was removed, dried over anhydrous magnesium sulphate, filtered and concentrated at reduced pressure to give the crude alkene intermediate as brown oil (ca. 6.7 g). TLC analysis (silica, ethyl acetate) showed that no (+)-β-DHTBZ remained in the crude product.

The crude alkene was taken up (dry nitrogen atmosphere) in anhydrous tetrahydrofuran (40 ml) and a solution of borane in THF (1 M solution, 2.5 eq, 52 ml) was added with stirring over fifteen minutes. The reaction mixture was then stirred at room temperature for two hours. TLC analysis (silica, ethyl acetate) showed that no alkene intermediate remained in the reaction mixture.

A solution of sodium hydroxide (3.7 g) in water (10 ml) was added to the stirring reaction mixture, followed by an aqueous solution of hydrogen peroxide (50%, ca. 7 ml) and the two-phase mixture formed was stirred at reflux for one hour. TLC analysis of the organic phase at this time (silica, ethyl acetate) showed the

appearance of a product with Rf as expected for Isomer B. A characteristic non-polar component was also seen.

The reaction mixture was allowed to cool to room temperature and was poured into a separating funnel. The upper organic layer was removed and concentrated under reduced pressure to remove the majority of THF. The residue was taken up in ether (stabilised (BHT), 75 ml), washed with water (40 ml), dried over anhydrous magnesium sulphate, filtered and concentrated under reduced pressure to give a pale yellow oil (8.1 g).

The yellow oil was purified using column chromatography (silica, ethyl acetate : hexane (80:20), increasing to 100% ethyl acetate) and the desired column fractions collected, combined and concentrated at reduced pressure to give a pale oil which was treated with ether (stabilised, 18 ml) and concentrated at reduced pressure to give Isomer B as a pale yellow solid foam (2.2 g).

Chiral HPLC using the conditions set out in Example 3B confirmed that Isomer B had been produced in an enantiomeric excess (e.e.) of greater than 97%.

The optical rotation was measured using a Bellingham Stanley ADP220 polarimeter and gave an $[\alpha_D]$ of +123.5°.

3D. Preparation of the Mesylate salt of Isomer B

The methanesulphonate salt of Isomer B was prepared by dissolving a mixture of 1 equivalent of Isomer B from Example 3C and 1 equivalent of methane sulphonic acid in the minimum amount of ethanol and then adding diethyl ether. The resulting white precipitate that formed was collected by filtration and dried *in vacuo* to give the mesylate salt in a yield of ca. 85% and a purity (by HPLC) of ca. 96%.

EXAMPLE 4

X-Ray Crystallographic Studies on Isomer B

The (*S*)-(+)-Camphor-10-sulphonic acid salt of Isomer B was prepared and a single crystal was subjected to X-ray crystallographic studies under the following conditions:

Diffractometer: Nonius KappaCCD area detector (t/i scans and OJ scans to fill asymmetric unit).

Cell determination: DirAx (Duisenberg, A.J.M.(1992). *J. Appl. Cryst.* 25, 92-96.)

Data collection: Collect (Collect: Data collection software, R. Hooft, Nonius B. V, 1998)

Data reduction and cell refinement: Demo (Z. Otwinowski & W. Minor, *Methods in Enzymology* (1997) Vol. 276: *Macromolecular Crystallography*, part A, pp. 307-326; C. W. Carter, Jr & R. M. Sweet, Eds., Academic Press).

Absorption correction: Sheldrick, G. M. SADABS - Bruker Nonius area detector scaling and absorption correction - V2.\ 0

Structure solution: SHELXS97 (G. M. Sheldrick, *Acta Cryst.* (1990) A46 467-473). Structure refinement: SHELXL97 (G. M. Sheldrick (1997), University of Göttingen, Germany)

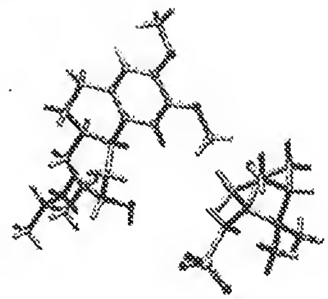
Graphics: Cameron - A Molecular Graphics Package (D. M. Watkin, L. Pearce and C. K. Prout, Chemical Crystallography Laboratory, University of Oxford,1993)

Special details: All hydrogen atoms were placed in idealised positions and refined using a riding model, except those of the NH and OH which were located in the difference map and refined using restraints. Chirality: NI=R, CI2=S, CI3=S, CI5=R, C21=S, C24=R

The results of the studies are set out below in Tables A, B, C, D and E.

In the Tables, the label RUS0350 refers to Isomer B.

TABLE A



Identification code	**2005bdy0585 (RUS0350)**
Empirical formula	$C_{29}H_{45}NO_7S$
Formula weight	551.72
Temperature	120(2) K
Wavelength	0.71073 Å
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
Unit cell dimensions	a = 7.1732(9) Å
	b = 12.941(2) Å
	c = 31.025(4) Å
Volume	2880.1(7) Å^3
Z	4
Density (calculated)	1.272 Mg / m^3
Absorption coefficient	0.158 mm^{-1}
F(000)	1192
Crystal	Colourless Slab
Crystal size	0.2 × 0.2 × 0.04 mm^3
θ range for data collection	3.06 – 27.37°
Index ranges	$-8 \le h \le 9$, $-16 \le k \le 16$, $-36 \le l \le 39$
Reflections collected	36802
Independent reflections	6326 [R_{int} = 0.0863]
Completeness to θ = 27.37°	97.1 %
Absorption correction	Semi–empirical from equivalents
Max. and min. transmission	0.9937 and 0.9690
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	6326 / 1 / 357
Goodness-of-fit on F^2	1.042
Final R indices [$F^2 > 2\sigma(F^2)$]	$R1$ = 0.0498, $wR2$ = 0.0967
R indices (all data)	$R1$ = 0.0901, $wR2$ = 0.1108
Absolute structure parameter	0.04(8)
Extinction coefficient	0.0059(7)
Largest diff. peak and hole	0.236 and –0.336 e Å^{-3}

TABLE B. Atomic coordinates [x 10^4], equivalent isotropic displacement parameters [A^2 x 10^3] and site occupancy factors. U_{eq} is defined as one third of the trace of the orthogonalized U^{ij} tensor.

Atom	x	y	z	Ueq	S.o.f.
N1	4839(3)	11119(2)	2180(1)	24(1)	1
O1	2515(3)	13171(1)	349(1)	31(1)	1
O2	5581(3)	14030(1)	598(1)	32(1)	1
O3	9220(3)	12834(2)	2385(1)	35(1)	1
C1	870(4)	12674(2)	190(1)	36(1)	1
C2	3176(3)	12838(2)	739(1)	25(1)	1

C3	2346(4)	12109(2)	997(1)	25(1)	1
C4	3124(3)	11821(2)	1395(1)	24(1)	1
C5	4773(3)	12276(2)	1527(1)	23(1)	1
C6	5629(4)	13024(2)	1262(1)	24(1)	1
C7	4861(4)	13308(2)	875(1)	25(1)	1
C8	7189(4)	14582(2)	747(1)	38(1)	1
C9	2182(3)	11023(2)	1673(1)	28(1)	1
CI0	2759(3)	11118(2)	2137(1)	26(1)	1
CII	5366(3)	11096(2)	2656(1)	25(1)	1
C12	7292(4)	11536(2)	2747(1)	25(1)	1
C13	7468(4)	12663(2)	2590(1)	25(1)	1
C14	5988(4)	12911(2)	2252(1)	25(1)	1
C15	5773(4)	12010(2)	1943(1)	24(1)	1
C16	7734(4)	11477(2)	3232(1)	28(1)	1
C17	7752(4)	10418(2)	3449(1)	34(1)	1
C18	9198(6)	9696(3)	3249(1)	65(1)	1
C19	8114(4)	10562(2)	3930(1)	41(1)	1
C20	7509(4)	8131(2)	1250(1)	31(1)	1
S1	7409(1)	8792(1)	1754(1)	27(1)	1
04	7758(2)	7965(1)	2064(1)	30(1)	1
05	8831(3)	9582(2)	1760(1)	49(1)	1
06	5524(2)	9221(1)	1798(1)	32(1)	1
07	7406(3)	6932(1)	498(1)	48(1)	1
C21	6858(3)	8622(2)	830(1)	25(1)	1
C22	7154(4)	7851(2)	459(1)	30(1)	1
C23	7073(4)	8450(2)	40(1)	32(1)	1
C24	6648(3)	9544(2)	203(1)	28(1)	1
C25	4742(3)	8877(2)	787(1)	29(1)	1
C26	4742(3)	8877(2)	787(1)	29(1)	1
C27	7773(4)	9610(2)	630(1)	25(1)	1
C28	7431(4)	10628(2)	868(1)	29(1)	1
C29	9895(4)	9489(2)	569(1)	36(1)	1

TABLE C. Bond lengths [A] and angles [°].

NI-CIO	1.498(3)	C14-C15	1.518(3)
NI-CI6	1.522(3)	C16-C17	1.526(3)
NI-CII	1.524(3)	C17-C18	1.527(4)
01-C2	1.368(3)	C17-C19	1.527(4)
OI-CI	1.432(3)	C20-C21	1.525(3)
02-C7	1.369(3)	C20-S I	1.784(2)
02-C8	1.433(3)	SI-05	1.4442(19)
03-C13	1.425(3)	SI-04	1.4607(17)
C2-C3	1.372(3)	SI-06	1.4676(18)
C2-C7	1.417(3)	07-C22	1.208(3)
C3-C4	1.407(3)	C21-C22	1.537(4)
C4-C5	1.384(3)	C21-C26	1.559(3)
C4-C9	1.506(3)	C21-C27	1.565(3)

C5-C6	1.411(3)	C22-C23	1.517(4)
C5-C15	1.516(3)	C23-C24	1.535(4)
C6-C7	1.372(3)	C24-C25	1.548(4)
C9-CI0	1.504(3)	C24-C27	1.554(4)
CII-CI2	1.521(3)	C25-C26	1.557(4)
C12-C16	1.540(3)	C27-C28	1.529(3)
C12-C13	1.544(3)	C27-C29	1.542(4)
C13-C14	1.524(3)		

CI0-NI-CI5	113.33(19)	CI2-CII-NI	113.43(19)
CI0-NI-CII	109.46(18)	CII-CI2-CI6	110.5(2)
CI5-NI-CII	111.96(19)	CII-CI2-CI3	111.7(2)
C2-01-CI	116.6(2)	CI6-CI2-CI3	109.84(19)
C7-02-C8	116.27(19)	03-CI3-CI4	106.0(2)
01-C2-C3	125.5(2)	03-CI3-CI2	111.1(2)
01-C2-C7	115.0(2)	CI4-CI3-CI2	111.0(2)
C3-C2-C7	119.5(2)	CI5-CI4-CI3	110.1(2)
C2-C3-C4	121.5(2)	C5-CI5-CI4	114.3(2)
C5-C4-C3	119.2(2)	C5-CI5-NI	112.0(2)
C5-C4-C9	120.3(2)	CI4-CI5-NI	108.7(2)
C3-C4-C9	120.5(2)	CI7-CI6-CI2	118.4(2)
C4-C5-C6	119.4(2)	CI6-CI7-CI8	112.2(2)
C4-C5-CI5	124.1(2)	CI6-CI7-CI9	108.7(2)
C6-C5-CI5	116.6(2)	CI8-CI7-CI9	110.8(3)
C7-C6-C5	121.3(2)	C21-C20-S1	122.51(18)
02-C7-C6	125.4(2)	05-SI-04	112.93(11)
02-C7-C2	115.4(2)	05-SI-06	112.47(12)
C6-C7-C2	119.2(2)	04-SI-06	111.93(11)
CI0-C9-C4	111.7(2)	05-SI-C20	108.81(13)
NI-CI0-C9	111.0(2)	04-SI-C20	102.60(11)

06-SI-C20	107 .44(12)	C23-C24-C25	108.4(2)
C20-C21-C22	109.0(2)	C23-C24-C27	103.3(2)
C20-C21-C26	117.3(2)	C25-C24-C27	102.3(2)
C22-C21-C26	102.1(2)	C24-C25-C26	102.9(2)
C20-C21-C27	123.4(2)	C25-C26-C21	104.2(2)
C22-C21-C27	100.21(19)	C28-C27-C29	107.8(2)
C26-C21-C27	101.7(2)	C28-C27-C24	112.0(2)
07-C22-C23	126.4(2)	C29-C27-C24	113.7(2)
07-C22-C21	125.9(2)	C28-C27-C21	116.5(2)
C23-C22-C21	107.7(2)	C29-C27-C21	112.3(2)
C22-C23-C24	101.3(2)	C24-C27-C21	94.27(19)

TABLE D. Anisotropic displacement parameters [A ^{2}x 10^3]. The anisotropic displacement factor exponent takes the form:- $2\pi^2[h^2 a^{*2} U^{11} + ... + 2\,h\,k\,a^*\,b^*\,U^{12}]$.

Atom	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}

NI	26(1)	24(1)	23(1)	2(1)	-1(1)	-3(1)
01	37(1)	30(1)	24(1)	3(1)	-7(1)	-4(1)
02	41(1)	31(1)	25(1)	5(1)	-2(1)	-10(1)
03	26(1)	49(1)	32(1)	7(1)	-3(1)	-9(1)
CI	41(2)	36(2)	32(2)	3(1)	-9(1)	-8(2)
C2	30(2)	24(2)	22(1)	1(1)	-1(1)	2(1)
C3	25(1)	26(1)	24(1)	-3(1)	-2(1)	2(1)
C4	26(2)	22(1)	23(1)	-1(1)	2(1)	-1(1)
C5	24(1)	22(1)	23(1)	-2(1)	1(1)	0(1)
C6	26(1)	22(1)	24(1)	-3(1)	2(1)	-5(1)
C7	30(2)	22(1)	22(1)	2(1)	4(1)	-4(1)
C8	45(2)	34(2)	36(2)	5(1)	-2(1)	-20(2)
C9	23(1)	32(1)	29(2)	3(1)	-1(1)	-4(1)
CIO	26(1)	29(1)	25(1)	2(1)	0(1)	-5(1)
C11	31(1)	25(1)	20(1)	2(1)	0(1)	-2(1)
C12	26(1)	26(1)	23(1)	-1(1)	1(1)	-1(1)
CI3	26(1)	28(1)	23(1)	-1(1)	-1(1)	-2(1)
CI4	30(2)	22(2)	24(1)	-1(1)	1(1)	-1(1)
CI5	22(1)	22(1)	28(1)	2(1)	0(1)	-4(1)
C16	31(1)	28(1)	24(1)	-1(1)	-3(1)	3(1)
CI7	46(2)	31(2)	25(1)	1(1)	-7(1)	0(2)
CI8	106(3)	46(2)	41(2)	6(2)	-1(2)	31(2)
C19	51(2)	41(2)	31(2)	9(2)	-7(1)	-4(2)
C20	30(2)	34(2)	29(1)	2(1)	3(1)	9(2)
S1	27(1)	30(1)	24(1)	4(1)	-2(1)	-5(1)
O4	31(1)	36(1)	23(1)	9(1)	-1(1)	0(1)
O5	53(1)	58(1)	37(1)	13(1)	-11(1)	-35(1)
O6	34(1)	35(1)	28(1)	-3(1)	-2(1)	10(1)
O7	81(2)	25(1)	40(1)	-1(1)	12(1)	6(1)
C21	26(1)	25(2)	24(1)	-1(1)	3(1)	2(1)
C22	35(2)	25(2)	31(2)	0(1)	1(1)	-1(1)
C23	40(2)	30(2)	25(1)	-2(1)	1(1)	-2(1)
C24	28(1)	29(2)	26(2)	2(1)	2(1)	2(1)
C25	30(2)	34(2)	29(2)	-1(1)	-2(1)	0(1)
C26	26(1)	34(2)	28(2)	0(1)	1(1)	-5(1)
C27	23(1)	26(1)	26(1)	0(1)	2(1)	0(1)
C28	31(1)	26(1)	30(1)	0(1)	-2(1)	-6(1)
C29	29(2)	41(2)	40(2)	0(2)	2(1)	-3(1)

TABLE E. Hydrogen coordinates [x 10^4] and isotropic displacement parameters [$Å^2$ x 10^3].

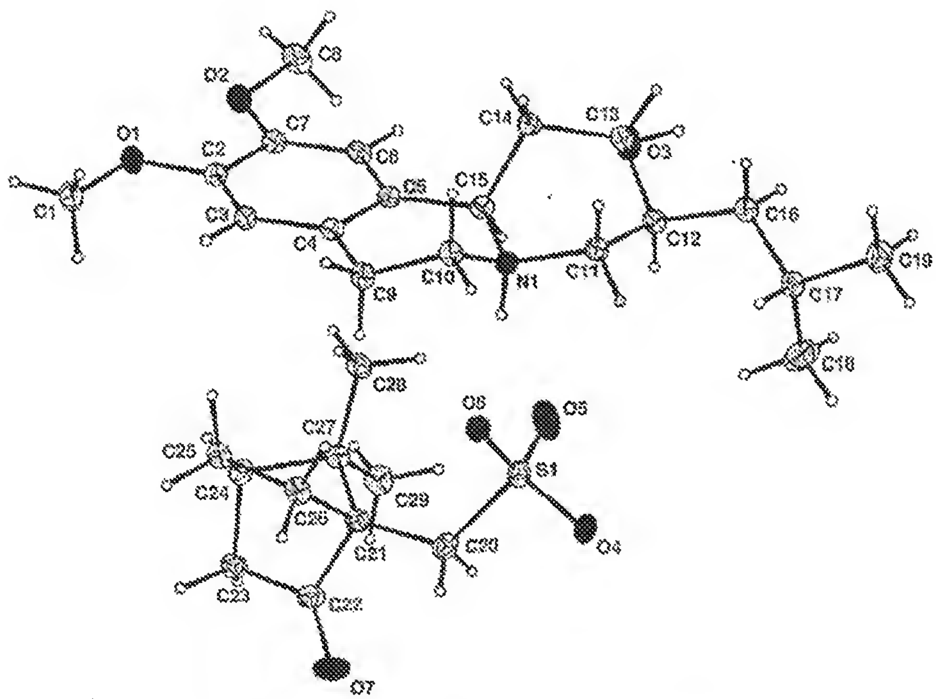
Atom	*x*	*y*	*z*	U_{eq}	*S.o.f*
H98	5190(40)	10528(15)	2062(10)	70(8)	1
H99	10030(50)	12950(30)	2575(12)	70(8)	1
H1A	1107	11933	156	54	1
H1B	529	12973	-89	54	1

H1C	-154	12777	395	54	1
H3	1220	11793	904	30	1
H6	6760	13337	1353	29	1
H8A	6872	14966	1009	58	1
H8B	7600	15065	523	58	1
H8C	8193	14091	810	58	1
H9A	814	11106	1651	33	1
H9B	2505	10324	1567	33	1
H10A	2250	11767	2259	32	1
H10B	2235	10534	2304	32	1
H11A	4431	11494	2822	30	1
H11B	5322	10372	2759	30	1
H12	8230	11108	2589	30	1
H13	7334	13145	2840	30	1
H14A	4783	13050	2397	30	1
H14B	6354	13538	2090	30	1
H15	7056	11776	1854	29	1
H16A	8973	11796	3278	33	1
H16B	6813	11911	3386	33	1
H17	6493	10098	3412	41	1
H18A	8906	9588	2944	97	1
H18B	9176	9031	3400	97	1
H18C	10440	10005	3276	97	1
H19A	9329	10894	3971	62	1
H19B	8110	9887	4073	62	1
H19C	7135	10999	4054	62	1
H20A	8824	7924	1207	37	1
H20B	6787	7484	1286	37	1
H23A	6070	8190	-151	38	1
H23B	8277	8423	-116	38	1
H24	6928	10107	-8	33	1
H25A	3773	9195	153	37	1
H25B	4152	10235	426	37	1
H26A	3994	8237	764	35	1
H26B	4300	9279	1039	35	1
H28A	8160	10638	1136	44	1
H28B	6103	10692	936	44	1
H28C	7811	11207	684	44	1
H29A	10358	10042	381	54	1
H29B	10159	8817	436	54	1
H29C	10517	9531	849	54	1

Table 6. Hydrogen bonds [Å and °].

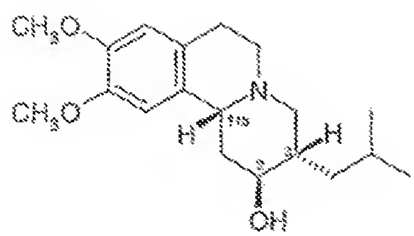
D–H···*A*	*d*(*D*–H)	*d*(H···*A*)	*d*(*D*···*A*)	∠(*DHA*)
N1–H98···O6	0.885(10)	1.895(12)	2.773(3)	171(3)
N1–H98···S1	0.885(10)	2.914(14)	3.771(2)	163(3)
O3–H99···O4^{i}	0.84(4)	1.94(4)	2.766(3)	165(3)
O3–H99···S1^{i}	0.84(4)	2.98(4)	3.811(2)	169(3)

Symmetry transformations used to generate equivalent atoms:
(i) −x+2,y+1/2,−z+1/2



Thermal ellipsoids drawn at the 30% probability level

On the basis of the data set out above, Isomer B is believed to have the 2S, 3S, 11bR configuration, which corresponds to Formula (Ia):



(Ia) – Isomer B

EXAMPLE 5

Receptor and Transporter Protein Binding Studies

The four dihydrotetrabenazine isomers A, B, C and D were subjected to specific binding assays to test their ability to bind to the receptors and transporter proteins described below. The results are set out in Table 5

(a) Adrenergic α_{2A} Receptor:

Reference:	S. Uhlen *et al. J. Pharmacol. Exp. Ther.*, 271:1558-1565 (1994)
Source:	Human recombinant insect Sf9 cells
Ligand:	1 nM [^{3}H] MK-912
Vehicle:	1% DMSO
Incubation time/Temp:	60 minutes @ 25 °C
Incubation buffer:	75mM Tris-HCl, pH 7.4, 12.5mM $MgCl_2$, 2mM EDTA
Non Specific ligand:	10µM WB-4101
K_d:	0.6 nM
B_{max}:	4.6 pmole/mg protein
Specific binding:	95%
Quantitation method:	Radioligand binding
Significance criteria:	≥ 50% of maximum stimulation or inhibition

(b) Adrenergic α_{2B} Receptor:

Reference:	S. Uhlen *et al.*, *Eur. J. Pharmacol.*, 33 (1): 93-1-1 (1998)
Source:	Human recombinant CHO-K1 cells
Ligand:	2.5 nM [3H] Rauwolscine

Vehicle:	1% DMSO
Incubation time/Temp:	60 minutes @ 25 °C
Incubation buffer:	50 mM Tris-HCl, 1 mM EDTA, 12.5mM $MgCl_2$, pH
7.4,	0.2% BSA at 25 °C
Non Specific ligand:	10 µM Prazosin
K_d:	2.1 nM
B_{max}:	2.1 pmole/mg protein
Specific binding:	90%
Quantitation method:	Radioligand binding
Significance criteria:	≥ 50% of maximum stimulation or inhibition

(c) Dopamine D_1 Receptor:

Reference:	Dearry *et al.*, *Nature*, 347:72-76, (1990)
Source:	Human recombinant CHO cells
Ligand:	1.4 nM [3H] SCH-23390
Vehicle:	1% DMSO
Incubation time/Temp:	2 hours @ 37 °C
Incubation buffer:	50 mM Tris-HCl, pH 7.4, 150 nM NaCl, 1.4 nM ascorbic acid, 0.001% BSA
Non Specific ligand:	10 µM (+)-butaclamol
K_d:	1.4 nM
B_{max}:	0.63 pmole/mg protein
Specific binding:	90%
Quantitation method:	Radioligand binding
Significance criteria:	≥ 50% of maximum stimulation or inhibition

(d) Dopamine D_{2L} Receptor:

Reference:	Bunzo *et al.*, *Nature*, 336:783-787 (1988)
Source:	Human recombinant CHO cells
Ligand:	0.16 nM [3H] Spiperone
Vehicle:	1% DMSO
Incubation time/Temp:	2 hours @ 25 °C

Incubation buffer:	50 mM Tris-HCl, pH 7.4, 150 nM NaCl, 1.4 nM ascorbic acid, 0.001% BSA
Non Specific ligand:	10 µM Haloperidol
K_d:	0.08 nM
B_{max}:	0.48 pmole/mg protein
Specific binding:	85%
Quantitation method:	Radioligand binding
Significance criteria:	≥ 50% of maximum stimulation or inhibition

(e) Dopamine D_3 Receptor:

Reference:	Sokoloff *et al.*, *Nature*, 347:146-151, (1990)
Source:	Human recombinant CHO cells
Ligand:	0.7 nM [3H] Spiperone
Vehicle:	1% DMSO
Incubation time/Temp:	2 hours @ 37 °C
Incubation buffer:	50 mM Tris-HCl, pH 7.4, 150 nM NaCl, 1.4 nM ascorbic acid, 0.001% BSA
Non Specific ligand:	25 µM S(-)-Sulpiride
K_d:	0.36 nM
B_{max}:	1.1 pmole/mg protein
Specific binding:	85%
Quantitation method:	Radioligand binding
Significance criteria:	≥ 50% of maximum stimulation or inhibition

(f) Imidazoline I_2 (Central) Receptor:

Reference:	Brown *et al.*, *Brit. J. Pharmacol.*, 99:803-809, (1990)
Source:	Wistar rat cerebral cortex
Ligand:	2 nM [3H] Idazoxan
Vehicle:	1% DMSO
Incubation time/Temp:	30 minutes @ 25°C
Incubation buffer:	50 mM Tris-HCl, 0.5 mM EDTA, pH 7.4 at 25 °C
Non Specific ligand:	1 µM Idazoxan
K_d:	4 nM

B_{max}:	0.14 pmole/mg protein
Specific binding:	85%
Quantitation method:	Radioligand binding
Significance criteria:	≥ 50% of maximum stimulation or inhibition

(g) Sigma σ_1 Receptor:

Reference:	Ganapathy *et al.*, *Pharmacol. Exp. Ther.*, 289:251-260, (1999)
Source:	Human jurkat cells
Ligand:	8 nM [3H] Haloperidol
Vehicle:	1% DMSO
Incubation time/Temp:	4 hours @ 25 °C
Incubation buffer:	5 mM K2HPO4/KH2PO4 buffer pH 7.5
Non Specific ligand:	10 µM Haloperidol
K_d:	5.8 nM
B_{max}:	0.71 pmole/mg protein
Specific binding:	80%
Quantitation method:	Radioligand binding
Significance criteria:	≥ 50% of maximum stimulation or inhibition

(h) Sigma σ_2 Receptor:

Reference:	Hashimoto *et al.*, *Eur. J. Pharmacol.*, 236:159-163, (1993)
Source:	Wistar rat brain
Ligand:	3 nM [3H] Ifenprodil
Vehicle:	1% DMSO
Incubation time/Temp:	60 minutes @ 37 °C
Incubation buffer:	50 mM Tris-HCl, pH 7.4
Non Specific ligand:	10 µM Ifenprodil
K_d:	4.8 nM
B_{max}:	1.3 pmole/mg protein
Specific binding:	85%
Quantitation method:	Radioligand binding

Significance criteria:	≥ 50% of maximum stimulation or inhibition

(i) Serotonin Transporter (SERT):

Reference:	Gu *et al.*, *J. Biol. Chem.*, 269(10):7124-7130, (1994)
Source:	Human recombinant HEK-293 cells
Ligand:	0.15 nM [125I] RTI-55
Vehicle:	1% DMSO
Incubation time/Temp:	3 hours @ 4 °C
Incubation buffer:	100 mM NaCl, 50 mM Tris HCl, 1 µM Leupeptin, 10 µM PMSF, pH 7.4
Non Specific ligand:	10 µM Imipramine
K_d:	0.17 nM
B_{max}:	0.41 pmole/mg protein
Specific binding:	95%
Quantitation method:	Radioligand binding
Significance criteria:	≥ 50% of maximum stimulation or inhibition

(j) Dopamine Transporter (DAT):

Reference:	Giros *et al.*, *Trends Pharmacol. Sci.*, 14, 43-49 (1993) Gu *et al.*, *J. Biol. Chem.*, 269(10):7124-7130 (1994)
Source:	Human recombinant CHO cells
Ligand:	0.15 nM [^{125}I] RTI-55
Vehicle:	1% DMSO
Incubation time/Temp:	3 hours @ 4 °C
Incubation buffer:	100 mM NaCl, 50 mM Tris HCl, 1 µM Leupeptin, 10 µM PMSF, pH 7.4
Non Specific ligand:	10 µM Nomifensine
K_d:	0.58 nM
B_{max}:	0.047 pmole/mg protein
Specific binding:	90%
Quantitation method:	Radioligand binding
Significance criteria:	≥ 50% of maximum stimulation or inhibition

(k) α_{2c} adrenergic receptor

α_{2c} Adrenergic receptor binding activity was determined using the method of Uhlen *et al., J. Pharmacol. Exp. Ther.* (1994), 271:1558-1565, and the following conditions:

Source:	Human recombinant insect Sf9 cells
Ligand:	1 nM [^{3}H] MK-912
Vehicle:	1% DMSO
Incubation time/Temp:	60 minutes @ 25 °C
Incubation buffer:	75mM Tris-HCl, pH 7.4, 12.5mM $MgCl_2$, 2mM EDTA
Non Specific ligand:	10µM WB-4101
K_d:	0.17 nM
B_{max}:	6.8 pmole/mg protein
Specific binding:	95%
Quantitation method:	Radioligand binding
Significance criteria:	≥ 50% of maximum stimulation or inhibition

(l) Serotonin (5-hydroxytryptamine) 5-HT$_{2b}$ receptor

5-HT$_{2b}$ receptor binding activity was determined using the method of Bonhaus *et al., Br. J. Pharmacol.*, (1995) 115:622-628, and the following conditions:

Source:	Human recombinant CHO-K1 cells
Ligand:	1.2 nM [^{3}H] lysergic acid diethylamide (LSD)
Vehicle:	1% DMSO
Incubation time/Temp:	60 minutes @ 37 °C
Incubation buffer:	50 mM Tris-HCl, pH 7.4, 4 mM $CaCl_2$, 0.1% ascorbic acid
Non Specific ligand:	10 µM serotonin
K_d:	2.1 nM
B_{max}:	1.1 pmole/mg protein
Specific binding:	80%
Quantitation method:	Radioligand binding
Significance criteria:	≥ 50% of maximum stimulation or inhibition

(m) Serotonin (5-hydroxytryptamine) 5-HT_6 receptor

5-HT_6 receptor binding activity was determined using the method of Monsma *et al.*, *Mol. Pharmacol.*, (1993), 43:320-327, and the following conditions:

Source:	Human recombinant HeLa cells
Ligand:	1.5 nM [^{3}H] lysergic acid diethylamide (LSD)
Vehicle:	1% DMSO
Incubation time/Temp:	2 hours @ 37 °C
Incubation buffer:	50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 2 mM ascorbic acid, 0.001% BSA
Non Specific ligand:	5 μM serotonin
K_d:	1.3 nM
B_{max}:	1.7 pmole/mg protein
Specific binding:	90%
Quantitation method:	Radioligand binding
Significance criteria:	≥ 50% of maximum stimulation or inhibition

Table 5

Percentage Inhibition by 10 μM Solutions of Dihydrotetrabenazine isomers of Specific Binding at Receptor and Transporter Proteins (IC_{50} value, where measured, is in parentheses)				
Receptor/Protein	Isomer A	Isomer B	Isomer C	Isomer D
(a) α_{2A} Receptor	86	12	13	87
(b) α_{2B} Receptor	44	14	-7	50
(c) D_1 Receptor	78	1	6	38
(d) D_{2L} Receptor	87	16	-14	58
(e) D_3 Receptor	69	7	9	63
(f) I_2 Receptor	74	8	0	55

Percentage Inhibition by 10 μM Solutions of Dihydrotetrabenazine isomers of Specific Binding at Receptor and Transporter Proteins (IC$_{50}$ value, where measured, is in parentheses)				
Receptor/Protein	Isomer A	Isomer B	Isomer C	Isomer D
(g) σ_1 Receptor	48	82	59	82
(h) σ_2 Receptor	64	64	61	69
(i) SERT	19	86 (0.35)	77 (2.75)	8
(j) DAT	3	4	-2	2
(k) α_{2c} receptor	56	-6	3	74
(l) 5-HT$_{2b}$ receptor	74	10	14	43
(m) 5-HT$_6$ receptor	51	10	10	41

On the basis of the binding data for Isomers A and D for the dopamine and serotonin receptors, and by analogy with the dopamine-serotonin binding profiles of known antipsychotic agents, it is envisaged that Isomers A and D will be useful in the treatment of psychosis, for example psychosis arising from or associated with schizophrenia.

EXAMPLE 6

Cognitive function and antipsychotics: An investigation into the efficacy of Isomer A to improve a cognitive deficit induced by sub-chronic PCP in the novel object recognition task

Cognition in Schizophrenia:

The major clinical unmet need in schizophrenia is the treatment of negative and cognitive symptoms, since even the latest generation of atypical antipsychotic drugs offers little improvement. Notably, a cognitive deficit in patients with schizophrenia is now recognised as a core part of the disorder, and is believed to have a significant bearing on the patient's recovery and re-integration into society.

There have been few attempts to model the cognitive disturbances in schizophrenia, although some of the more recent, and arguably more valid animal models show cognitive deficits. The classical approaches used to provide animal models for the testing of potential antipsychotics have relied on the use of dopaminergic drugs, the limitations of which are increasingly being recognised. Administration of the glutamate/NMDA antagonist phencyclidine (PCP) has been considered to provide a better model of schizophrenia in that it can induce both negative symptoms as well as the positive symptoms associated with amphetamine psychosis (J. D. Jentsch and R. H. Roth, *Neuropsychopharmacology* (1999) 20(3): 201-225). This approach may have some pathological validity in that there is evidence of abnormalities of glutamatergic systems in the brain in schizophrenia; such changes include deficits in cortico-striatal innervation that may contribute to, if not underlie, cognitive dysfunction in the disease (Aparicio-Legarza *et al.*, *Neurosci. Lett.* (1997) 232, 13-16). In addition, some PCP-induced behaviours are reversed by certain atypical, but not typical antipsychotics (Geyer *et al.*, *Brain Res. Bull.* (1990) 25: 485-498.). This suggests a potential correlation with effects on negative or other symptoms that respond less well to the typical drugs.

The novel object recognition paradigm:

Certain pre-clinical tests allow the observation of relatively subtle cognitive deficits in the rat that resemble cognitive symptoms in subjects with schizophrenia. The cognitive deficits observed are seen in behaviours such as working memory deficits which may be measured by recognition tasks such as the novel object recognition (NOR) paradigm. A recognition memory task allows the comparison between presented stimuli and previously stored information. Ennaceur & Delacour, *Behav. Brain Res.* 31: 47-59 (1988) described the NOR test in rats which was based on the differential exploration of familiar and new objects. The NOR test is a non-rewarded, ethologically relevant paradigm based on the spontaneous exploratory behaviour of rats which measures working memory. Each session consists of two trials. In the first trial, the rats are exposed to two identical objects in an open field. During the second trial, rats are exposed to two dissimilar objects, one familiar object from the first trial and one new object. Object recognition in rats can be measured as the difference in time spent exploring the familiar and the new object.

Rats have been shown to spend more time exploring the new object. It was found that rats are able to discriminate between the familiar and the novel object when the inter-trial interval is between 1 minute and 1-5 hours, but not when it is greater than 24 hours, although this effect may be sex dependent in the rat (Sutcliffe et al, *A preliminary investigation into the effects of gender on cognition in male and female rats using the novel object recognition paradigm*. Presented at the 96th meeting of the Society for Endocrinology, 7-9th November 2005). The duration of each trial is also important as a preference for the novel object only lasts during the first 1 or 2 minutes, after which time preference diminishes as both objects become familiar and are explored equally.

(Grayson and Neill, *J. Psychopharmacology* 18: A55, 2004; and Proceedings of the BPS at http://wwwpA2online.org/vol 12issue4-abst077P.html. 2005) have demonstrated a selective deficit in this task induced by acute and sub-chronic treatment with PCP. The deficit is only observed in the retention phase of the task, suggesting a specific and relatively subtle cognitive impairment. Thus behaviour in the acquisition phase of the test (and locomotor activity) is unaffected by PCP treatment. The effects of PCP in this paradigm may represent a selective deficit in working memory which is known to be impaired in schizophrenia. J. C. Neill's group at the University of Bradford, United Kingdom, have found that the atypical antipsychotic drug clozapine, but not the classical antipsychotic, haloperidol, can ameliorate (and prevent, Idris *et al. Soc. Neurosci.* abstr. 67.15.2005) the cognitive deficit induced by sub-chronic PCP (2mg/kg ip twice daily for 7 days followed by 7 days drug-free period) in this paradigm. Haloperidol is known to be ineffective in the treatment of cognitive deficit symptoms in schizophrenia, and indeed may make them worse, while atypical antipsychotics can improve certain aspects of cognition in schizophrenia. Furthermore, Grayson *et al*, have recently demonstrated efficacy of risperidone to attenuate the sub-chronic PCP-induced deficit in this paradigm. Thus this test has some predictive validity for the treatment of cognitive symptoms of schizophrenia. The sub-chronic PCP-induced deficit has been shown to be robust and long-lasting in female rats, i.e. up to 5 months post-treatment.

Object of the Experiment

The abovementioned rodent model was used to assess the effects of the Isomer A on sub-chronic PCP-induced deficits in working memory using the novel object recognition (NOR) paradigm. The working hypothesis was that both acute and sub-chronic treatment with Isomer A will attenuate the selective working memory deficit induced by sub-chronic PCP as measured in the NOR test paradigm. Female rats were used in this paradigm as it has previously been found that males to be less sensitive to the deficit induced by PCP (Grayson and Neill, *idem.*) and females show more robust performance following increasing inter-trial intervals compared with male rats (Sutcliffe *et al*, *idem*).

Methods

The Novel Object Recognition Paradigm:

Habituation.

Rats are allowed to habituate to the empty test box and the behavioural test room environment for 1 hour on day 1. Prior to behavioural testing on day 2 rats are given a further 3 minutes habituation.

Behavioural testing.

Following the 3 minute habituation period, the rats are given two 3 minute trials (T1 and T2) which are separated by a 1 minute inter-trial interval in the home cage during which the objects are changed.

T1=Trial 1, the acquisition trial.

In this trial, the animals are allowed to explore two identical objects (A1 and A2) for 3 minutes.

T2=Trial 2, the retention trial.

In this trial, the animals explore a familiar object (A) from T1 and a novel object (B) for 3 minutes. The familiar object presented during T2 is a duplicate of the object presented in T1 in order to avoid any olfactory trails.

Object exploration.

The object exploration is defined by animals licking, sniffing or touching the object with the forepaws whilst sniffing, but not leaning against, turning around, standing or sitting on the object. The exploration time (s) of each object (A1, A2, A and B) in each trial are recorded using two stopwatches and the following factors are calculated:

- Total exploration time of both objects in the acquisition trial (s).
- Total exploration time of both objects in the retention trial (s).
- Habituation of exploratory activity. The LMA includes the exploration time, as measured by the number of lines crossed, for both the trials.
- Discrimination index, which is calculated as shown below;

(time spent exploring novel object – time spent exploring familiar object) ÷ total time spent in exploring the objects

Behaviour in all trials was recorded on video for subsequent blind scoring.

Subjects

50 female hooded-Lister rats (Harlan, UK) were used as subjects for these studies. Rats were housed in groups of 5 under standard laboratory conditions under a 12hr light:dark cycle, lights on at 0700 hr. All testing was carried out in the light phase. Food and water were freely provided. All experiments were conducted in accordance with the Animals Scientific Procedures Act, U.K. 1986 and were approved by the University of Bradford ethical review panel.

Drugs

Rats were randomly assigned to two treatment groups and treated with vehicle, n=10 (distilled water, ip) or PCP, n=40 (2 mg/kg, ip) twice daily for 7 days. Phencyclidine hydrochloride (PCP, Sigma, UK) was dissolved in distilled water. This was followed by a 7 day wash out period before the rats were tested. Isomer A was dissolved in distilled water and administered via the oral route at doses of 3, 10 and 30 mg/kg, 30 minutes prior to testing. Risperidone (0.2 mg/kg) was prepared in distilled water and injected i.p. 30 minutes prior to testing. All drugs were administered in a volume of 1ml/kg. All drug doses were calculated as base equivalent weight.

<u>Statistical Analysis</u>

All data are expressed as mean ± s.e.m (n=7-10 per group) and were analysed by a two way ANOVA (factors are; drug and exploration time of the two objects) with further analysis by a post-hoc student's t-test (time spent exploring objects) or Dunnett's t-test (LMA and DI).

<u>Drug treatment</u>

Groups of rats (n=7-10) were tested in the NOR paradigm as described above. Rats were tested for their performance in the task following sub-chronic treatment with PCP (2 mg/kg i.p. twice daily for 7 days followed by 7 days drug-free period) or vehicle followed by acute treatment with Isomer A, risperidone or vehicle. Rats were randomly assigned to the drug treatment groups and received vehicle or Isomer A (3.0, 10 and 30mg/kg) p.o. 30 minutes prior to behavioural testing.

<u>Results</u>

The results are shown in Figures 1 to 4.

Figure 1 illustrates the mean exploration time of identical objects in the acquisition phase-T1- following acute administration of Isomer A (3.0-30 mg/kg, p.o) and risperidone (Risp 0.2mg/kg, i.p) in sub-chronic PCP (2mg/kg, i.p twice daily for seven days) and vehicle treated rats.

Figure 2 illustrates the ability of acute Isomer A (3-30mg/kg, p.o) and risperidone (Risp 0.2mg/kg, i.p) to attenuate the effect of sub-chronic PCP on the exploration time (s) of a familiar object and a novel object in a 3 minute retention trial in female hL rats. Significant difference between time spent exploring the familiar and novel object *P<0.05-***P<0.001.

Figure 3 illustrates the effect of Isomer A (3-30 mg/kg, p.o.) and risperidone (Risp 0.2mg/kg, i.p.) on the effect of sub-chronic PCP (2mg/kg, i.p twice daily for seven days) treatment on the discrimination index (DI).

Figure 4 illustrates the effect of acute administration (3-30 mg/kg, p.o.) of Isomer A and risperidone (Risp 0.2mg/kg, i.p) in sub-chronically PCP treated rats on the total number of line crossings in the novel object recognition task (T1+T2). **p<0.01; significant reduction in number of line crossings compared with the vehicle control group.

Acute PCP (0.5-2.0mg/kg ip) and sub-chronic PCP (2mg/kg i.p. twice daily for 7 days followed by 7 days drug-free period) produce a selective cognitive deficit in the retention phase of the NOR task in female rats (Grayson and Neill, 2004; 2005a). The atypical antipsychotic agent clozapine (1-5mg/kg), but not haloperidol (0.05-0.075mg/kg) significantly improved (and prevented, Idris et al, 2005) the deficit induced by sub-chronic PCP in this paradigm (Grayson and Neill, 2005a). The present results add to this existing data and show that Isomer A also has efficacy to attenuate the sub-chronic PCP-induced deficit in a manner similar to the atypical antipsychotic, risperidone.

The effects of acute treatment with Isomer A were selective for the retention phase of the NOR task (Figure 2). Its effects are consistent with improvement of working memory deficits induced by PCP in a paradigm with some validity for the pathology of schizophrenia. This effect was significant at the highest dose of Isomer (30mg/kg). In contrast, Isomer A had no effect on exploration of two identical objects in the acquisition phase of the task, Figure 1. 30 mg/kg of Isomer A also had a significant effect to reduce locomotor activity in the test arena, Figure 4. This was shown as a reduction in the number of lines crossed in the novel object arena in T1 and T2. Observation of the behaviour of the rats suggested that they spent more time in object than environment exploration which reduced their overall activity score in the box. They did not appear sedated. Data shown in Figure 3 show that sub-chronic PCP treatment induced a reduction in the discrimination index, and that this was improved following 30 mg/kg of Isomer A and 0.2mg/kg of risperidone: however, none of these effects reached statistical significance.

The results set out herein suggest that Isomer A may have some therapeutic value in improvement of cognitive deficit symptoms of schizophrenia.

EXAMPLE 7

Pharmaceutical Compositions

(i) Tablet Formulation - I

A tablet composition containing a dihydrotetrabenazine of the invention is prepared by mixing 50 mg of the dihydrotetrabenazine with 197 mg of lactose (BP) as diluent, and 3 mg magnesium stearate as a lubricant and compressing to form a tablet in known manner.

(ii) Tablet Formulation - II

A tablet composition containing a dihydrotetrabenazine of the invention is prepared by mixing the compound (25 mg) with iron oxide, lactose, magnesium stearate, starch maize white and talc, and compressing to form a tablet in known manner.

(iii) Capsule Formulation

A capsule formulation is prepared by mixing 100 mg of a dihydrotetrabenazine of the invention with 100 mg lactose and filling the resulting mixture into standard opaque hard gelatin capsules.

Equivalents

It will readily be apparent that numerous modifications and alterations may be made to the specific embodiments of the invention described above without departing from the principles underlying the invention. All such modifications and alterations are intended to be embraced by this application.

CLAIMS

1. The use of 3,11b-*cis*-dihydrotetrabenazine or a pharmaceutically acceptable salt thereof for the manufacture of a medicament for the prophylaxis or treatment of schizophrenia.

2. The use of 3,11b-*cis*-dihydrotetrabenazine or a pharmaceutically acceptable salt thereof for the manufacture of a medicament for the prophylaxis or treatment of psychosis.

3. A compound for use in the prophylaxis or treatment of psychosis, the compound being 3,11b-*cis*-dihydrotetrabenazine or a pharmaceutically acceptable salt thereof.

4. A compound for use in preventing or alleviating psychosis, the compound being 3,11b-*cis*-dihydrotetrabenazine or a pharmaceutically acceptable salt thereof.

5. A method for the prophylaxis or treatment of psychosis, the method comprising administering to the mammal a therapeutically effective amount of *cis*-dihydrotetrabenazine or a pharmaceutically acceptable salt thereof.

6. The use of 3, 11b-*cis*-dihydrotetrabenazine or a pharmaceutically acceptable salt thereof for the manufacture of a medicament for preventing or alleviating a psychotic episode.

7. A method for preventing or alleviating a psychotic episode, the method comprising administering to the mammal a therapeutically effective amount of *cis*-dihydrotetrabenazine or a pharmaceutically acceptable salt thereof.

8. A compound for use, method or use as defined in any one of the preceding claims wherein the psychosis or psychotic episode arises from or is associated with schizophrenia.

9. A compound for use, method or use as defined in any one of the preceding claims wherein the psychotic episodes, psychoses or symptoms prevented, alleviated or reduced are selected from:

- delusions;
- hallucinations;
- visual hallucinations;
- auditory hallucinations;
- hallucinations involving tactile sensations, tastes or smells;
- confusion;
- emotional, behavioral, or intellectual disturbances;
- withdrawal from reality;
- illogical and/or disorganized patterns of thinking;
- paranoid or delusional beliefs;
- paranoia
- grandiose delusions;
- persecutory or self-blaming delusions; and
- personality changes.

10. A compound for use, method or use as defined in any one of the preceding claims wherein the psychotic episodes, psychoses or symptoms prevented, alleviated or reduced are selected from those arising from or associated with:

- psychosis caused by or associated with schizophrenia;
- psychosis caused by or associated with bipolar disorder (manic depression);
- psychosis caused by or associated with severe clinical depression;
- psychosis induced by disorders and conditions such as:
    - electrolyte disorder;
    - urinary tract infections in the elderly;
    - pain syndromes;
    - drug toxicity;
    - drug withdrawal; and
    - infections of or injuries to the brain;
- psychosis caused by chronic psychological stress (brief reactive psychosis);

- psychosis triggered or exacerbated by severe mental stress; and
- psychosis triggered by or arising from or following illnesses and conditions such as AIDS, leprosy, malaria and mumps.

11. A compound for use in the prophylaxis or treatment of schizophrenia, the compound being 3,11b-*cis*-dihydrotetrabenazine or a pharmaceutically acceptable salt thereof.

12. A compound for use in preventing, alleviating or reducing one or more symptoms of schizophrenia, the compound being 3,11b-*cis*-dihydrotetrabenazine or a pharmaceutically acceptable salt thereof.

13. A method for the prophylaxis or treatment of schizophrenia, the method comprising administering to the mammal a therapeutically effective amount of *cis*-dihydrotetrabenazine or a pharmaceutically acceptable salt thereof.

14. The use of 3, 11b-*cis*-dihydrotetrabenazine or a pharmaceutically acceptable salt thereof for the manufacture of a medicament for preventing, alleviating or reducing one or more symptoms of schizophrenia.

15. A method for preventing, alleviating or reducing one or more symptoms of schizophrenia, the method comprising administering to the mammal a therapeutically effective amount of *cis*-dihydrotetrabenazine or a pharmaceutically acceptable salt thereof.

16. A compound for use, use or method according to any one of claims 10 to 15 wherein the *cis*-dihydrotetrabenazine is administered for the purpose of preventing, alleviating or reducing one or more symptoms selected from:
    - delusions;
    - hallucinations;
    - confusion;
    - emotional, behavioral, or intellectual disturbances;
    - withdrawal from reality; and
    - illogical patterns of thinking.

17. A compound for use, use or method as defined in any one of claims 1 to 16 wherein the 3,11b-*cis*-dihydrotetrabenazine is Isomer A as defined herein.

18. A compound for use, use or method as defined in any one of claims 1 to 16 wherein the 3,11b-*cis*-dihydrotetrabenazine is Isomer D as defined herein.

19. A compound for use, use or method as defined in any one of claims 1 to 18 wherein the 3,11b-*cis*-dihydrotetrabenazine is in the form of an acid addition salt.

20. A compound for use, use or method as defined in claim 19 wherein the salt is a methane sulphonate salt.

21. A compound for use, use or method substantially as described herein with reference to the examples.

FIGURE 1

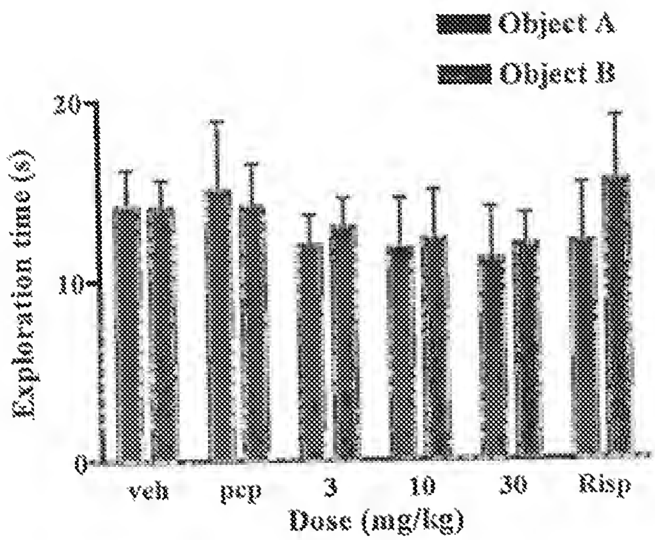


FIGURE 2

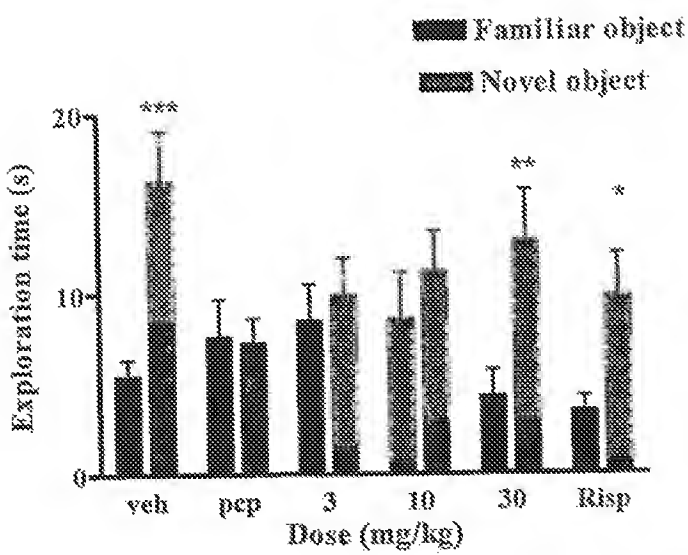


FIGURE 3

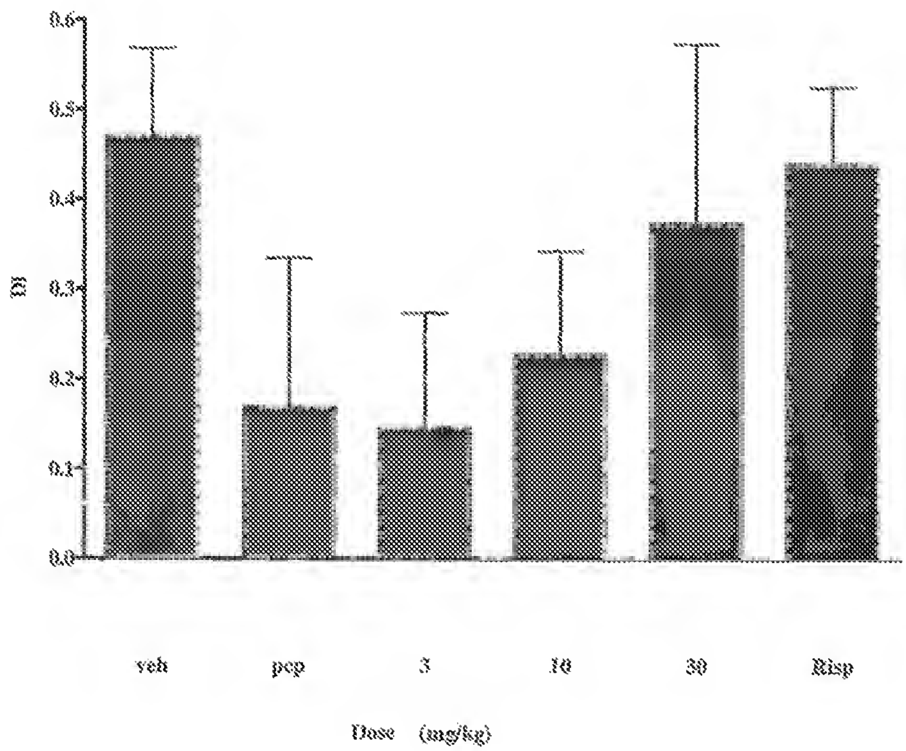
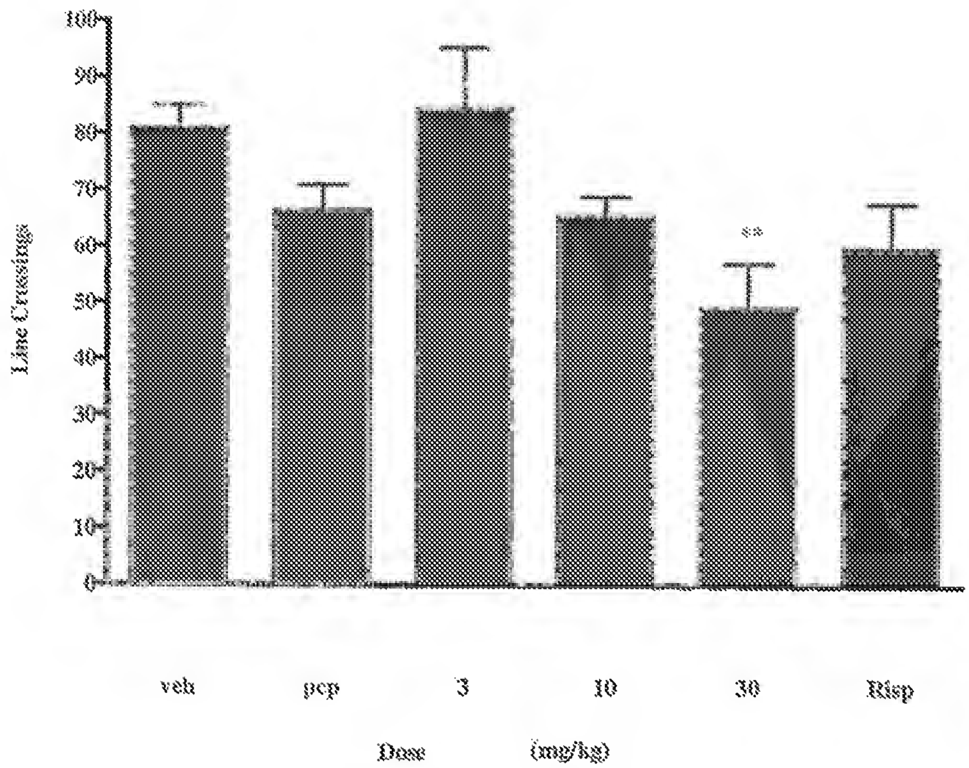


FIGURE 4


100
90
80
70
60
50
40
30
20
10
0
Line Crossings
**
veh
pcp
3
10
30
Risp
Dose (mg/kg)

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2006/002936

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/473 A61P25/18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MEHVAR R ET AL: "CONCENTRATION-EFFECT RELATIONSHIPS OF TETRABENAZINE AND DIHYDROTETRABENAZINE IN THE RAT" JOURNAL OF PHARMACEUTICAL SCIENCES, vol. 76, no. 6, 1987, pages 461-465, XP009075267 ISSN: 0022-3549 last paragraph	1-21
P,X	WO 2005/077946 A (CAMBRIDGE LAB LTD [GB]; TRIDGETT ROBERT [GB]; CLARKE IAN [GB]; TURTLE) 25 August 2005 (2005-08-25) cited in the application tables 1-5	3,4, 8-12, 16-21

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 December 2006	02/01/2007

Name and mailing address of the ISA/	Authorized officer
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International application No.
PCT/GB2006/002936

INTERNATIONAL SEARCH REPORT

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 5, 7, 13, 15 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/GB2006/002936

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2005077946 A	25-08-2005	AU 2005213525 A1	25-08-2005
		CA 2555815 A1	25-08-2005
		EP 1716145 A1	02-11-2006